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REPORT OF A NATIONAL INSTITUTES OF HEALTH WORKSHOP

Methods and Welfare Considerations in Behavioral Research with Animals

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Background

Behavioral research has made significant contributions to the understanding, treatment, and prevention of behavioral disorders. Experimental animals play an essential role in this work. The National Institute of Mental Health (NIMH), together with other institutes of the National Institutes of Health (NIH) that have relevant research programs, prepared this handbook. The handbook provides a description of and references for commonly used behavioral research methods and associated animal welfare considerations in accordance with Federal laws governing animal research. It is intended to assist Institutional Animal Care and Use Committees (IACUCs) in their reviews of protocols involving animal behavior and animal cognition, particularly when expertise is not available on the committee, and to assist investigators in planning their experiments.

The development of this handbook took place in three stages. Drs. Adrian Morrison and Richard Nakamura, in consultation with Drs. Hugh Evans and Steven Maier, representing the Committee on Animal Research and Ethics of the American Psychological Association, determined the general subject areas that this handbook would include. Research scientists with specific expertise in each area were selected to work with a section chairperson in creating a preliminary document that was presented at a 1-1/2-day conference. Present at the conference were participating researchers, laboratory animal veterinarians, and representatives from the United States Department of Agriculture (USDA), the Office of Laboratory Animal Welfare (OLAW), and the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC). Each chairperson was responsible for preparation of a document summarizing the salient points from each topic. The editors then incorporated revisions as provided by the reviewers. They also contributed substantially to the original writing in most of the chapters.

These conference documents served as the resource from which this volume was assembled and edited by Adrian Morrison, Nancy Ator, Hugh Evans, and Richard Nakamura with the editorial assistance of Deborah Faryna, employing the suggestions received from a wide range of commentators, including research scientists, laboratory animal veterinarians, and interested lay people. The document cannot provide a thorough review of the literature; it is meant to guide the researcher and IACUC to appropriate considerations and entry points in the literature. A few key references for various parts of this work are provided in the text. References are provided at the end of each chapter. **In addition to articles specifically mentioned in the text, there are additional references for further exploration of the issues.** Also, the reader should be assured that all statements, whether documented

specifically with a reference or not, are the words of experts in their fields that have been reviewed by laboratory animal veterinarians to ensure that welfare considerations are included. **IACUCs may wish to consider the contributors to this volume when seeking an outside expert for a particular protocol.**

Because the field is constantly evolving, and because of space limitations for this type of introductory volume, this document could not possibly be exhaustive. **Omission of any particular procedure should not be taken to mean that it is unacceptable.** We hope this volume can provide additional background and context for both researchers and IACUCs as they consider animal welfare issues with respect to individual research protocols. ■

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CHAPTER 1

Introduction

Understanding normal and abnormal behavior requires the study of living organisms. The evolution of organisms means that the study of a variety of animals has shed light on normal and abnormal behavior of humans, who are also animals, of course, in terms of their biology. Behavioral research has contributed significantly to the understanding, treatment, and prevention of behavioral and brain disorders. Animals as experimental models provide a continuity of psychological and biological information across species. Because of this continuity, use of animals in research that employs behavioral techniques has led to many advances in knowledge that benefit humans and animals (Miller, 1985). Examples of the contributions of animal research to human welfare are provided in Chapter 2, Contributions of Behavioral Research with Animals, as well as in the subsequent chapters dealing with specific methodologies.

Despite an impressive record of contribution and progress, the methodology and rationales of behavioral research sometimes are not well understood, which can be problematic for those reviewing behavioral research protocols. The relatively lengthy periods of time over which behavioral experiments are usually conducted, coupled with the need for precise control of environmental conditions to ensure valid and reliable outcomes, raise animal welfare considerations that often are different from, but no less important than, those raised by non-behavioral biomedical research.

Federal regulations and policies require institutional oversight of experiments using animal subjects to ensure that research animals are cared for properly. At the heart of the local compliance process is the IACUC, which ultimately determines the appropriate balance between the progress of biomedical and behavioral science and the welfare of the animals used for that progress. Diversity of research interests in an institution inevitably means that appropriate expertise relative to a particular field may be lacking on the committee. Thus, one of the most important actions a committee can take, and one that is recognized in the USDA animal welfare regulations and the United States Public Health Service (USPHS) Policy for the Humane Care and Use of Laboratory Animals, is the solicitation of expert opinion, not only with regard to the scientific question but also about the accumulated wisdom on the behavioral characteristics of various species (USDA, http://www.nal.usda.gov/awic/legislat/

usdaleg1.htm; USPHS, 1996). This facilitates a productive, cooperative climate at the institution as well as a more in-depth consideration of animal welfare issues.

At the same time, investigators recognize that progress in veterinary medicine brings advances to the laboratory that can improve an animal's health and welfare and the success rate of a particular experimental approach. Nevertheless, the principal investigator's training and track record should be considered when committees and veterinarians evaluate the proposals. Those with extensive experience may well be the most knowledgeable consultants about the behavioral needs and capabilities of a particular species. Long experience of investigators with a particular technique or preparation can provide insights into the type of care that is most appropriate, particularly for uncommon species or highly specialized research. Conversely, investigators who have conducted similar experiments for many years may benefit from being apprised of advances in fields that can enhance their research. In other words, all partners in the enterprise must be willing to acknowledge the limits of their expertise and to be open to additional sources of information.

Science demands investigation at the edges of human knowledge. This means that the ability to innovate and to ask questions for which the answer is not known is necessary for scientific progress. The USDA animal welfare regulations, the USPHS Policy, and the Institute for Laboratory Animal Research *Guide for the Care and Use of Laboratory Animals* (ILAR, 1996) all allow IACUCs to permit exceptions to guidelines under certain circumstances and if appropriately justified. This handbook, therefore, is intended to suggest factors that IACUCs can take into consideration when reviewing protocols for research to avoid being unnecessarily restrictive. Of course, no set of standards, guidelines, or considerations can be viewed as fixed: New circumstances, knowledge, and values must be incorporated into our judgments.

Each IACUC has to make an informed decision in all cases as to when a study may be at the limits of what is considered acceptable. Questions to be answered in these circumstances: Are there alternatives? Can the study be refined to reduce pain or distress further or to reduce the number of animals? If not, can the proposed study provide an answer to an important question?

Finally, both investigators and IACUCs should be aware of public perceptions and of the public's need to be educated by informed explanations on the use of animals. Research on animals is conducted largely through public support, financially and politically. This involves a level of trust that can be maintained only if information on the appropriateness, the benefits, and the attention to animal welfare that go into animal research is readily available and acceptable. ■

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United States Department of Agriculture website, http://www.nal.usda.gov/awic/legislat/usdaleg1.htm.

CHAPTER 2

Contributions of Behavioral Research with Animals

The excellent review by Neal Miller (1985), who has contributed so much to the advancement of behavioral research with his own work and efforts at public education provided an invaluable historical framework for the discussions in this chapter on fundamental contributions of behavioral research.

IACUC members understand, of course, that basic research may not have as immediately definable an outcome in terms of benefits to humans as applied research might, but that it is nevertheless of fundamental importance. The course of science has repeatedly shown how basic research serves as the cornerstone for applied developments. For example, basic research conducted over the past four decades by Arvid Carlsson, Paul Greengard, and Eric Kandel, who shared the 2000 Nobel Prize for Physiology or Medicine, provided the knowledge that has already borne fruit in the form of treatments for Parkinson's disease and drugs for use against schizophrenia and depression and may soon lead to treatments for Alzheimer's disease (Byrne, 2001).

Among other benefits basic behavioral research has achieved are (1) knowledge of basic learning processes and motivational systems; (2) understanding of the effects of social deprivation and appreciation of the value of environmental enrichment for the brain; (3) awareness that there is plasticity even in the adult brain; (4) knowledge of the central processing of vision and audition, diagnosis, and treatment of sleep disorders; and (5) appreciation for the neural underpinnings of drug addiction and alcoholism.

Many university-level students enroll in an introductory psychology course that discusses these topics. Yet, sadly, a study of a group of major textbooks revealed that "major findings from animal research were frequently presented as if they had been obtained with humans" (Domjan and Purdy, 1995). We believe, as well, that there is a general lack of appreciation for the critical role behavioral research has played in advancing human and animal welfare. Therefore, we have reviewed some of these achievements below for those who serve on IACUCs but may not be behavioral scientists. Many more examples may be found in *Animal*

Research and Human Health: Advancing Human Welfare Through Behavioral Science (Carroll and Overmier, 2001).

The route to major medical advances is tortuous and full of surprises. Perhaps there is no clearer example of this complexity than that provided by the development of psychotropic drugs. Chlorpromazine, for example, revolutionized the treatment of schizophrenia and truly alleviated human misery (Swazey, 1974). As Kety (1974) notes in his foreward to Swazey's book:

> One conclusion, immediately apparent and rather surprising, is that none of the crucial findings or pathways that led, over the course of a century, to the ultimate discovery of chlorpromazine would at first have been called relevant to the treatment of mental illness by even the most sophisticated judge. If scientists had decided in the middle of the last century [19th] to target research toward the treatment of schizophrenia, if they had been able to organize such a program, and if they had engaged the greatest minds, which of those crucial discoveries and pathways would they have supported as relevant to their goal? Certainly not the synthesis of phenothiazine by a chemist interested in methylene blue; nor the study of anaphylaxis in guinea pigs (which is more clearly related to asthma)...nor the study of the role of histamine in allergy and anaphylaxis and the search for antihistaminic drugs...*nor the studies on operant conditioning in animals* [editors' emphasis]; and not the search by an anesthesiologist for an antihistaminic-sympatholytic drug that might be useful in mitigating surgical shock.

Of course, the development and testing of subsequent drugs that have helped so many of the mentally ill have relied heavily on laboratory animals.

ANIMAL WELFARE

Behavioral research on animals has benefited animals as well as humans. For the past 15 years greater attention to the quality of the environment in which research and zoo animals live has resulted in improved animal welfare and more refined animal models for research. Increased environmental complexity, generally referred to as environmental enrichment, has been shown to influence brain development (Walsh, 1981), memory, learning ability (e.g., Escorihuela et al., 1995), and problem-solving; to mitigate some of the effects of undernutrition and old age; to promote recovery from brain trauma (Van Rijzingen, 1995); to improve the reproductive success of captive animals (Carlstead and Shepherdson, 1994) and alter the development of atherosclerosis; and to decrease the expression of abnormal behaviors while increasing the diversity of normal behaviors exhibited (Bayne et al., 1991; Duke, 1989; Gilloux et al., 1992; van de Weerd et al., 1997), thereby enhancing the psychological and physiological welfare of the animals.

Similarly, knowledge gained through research on animal behavior has proved invaluable for the successful reintroduction of captive-born animals into the wild (Castro et al., 1998; Miller

et al., 1998; Shepherdson, 1994) and for improving the lives of animals in zoos (Markowitz, 1982; Shepherdson, 1998). Understanding preferences, similarities, and differences among different species in their requirements for habitat, territory, and social interactions has greatly enhanced the welfare of these animals.

REHABILITATION MEDICINE

Nobel Laureate Charles Sherrington and his colleague (Mott and Sherrington, 1895) showed that sensory deafferentation—cutting the dorsal roots of the nerves supplying a forelimb—caused animals to stop using that limb. Later, behavioral research demonstrated that appropriate motivation could "rehabilitate" the deafferented forelimb to function without sensory feedback from the affected limb (Taub et al., 1965).

Taub and his colleagues have since demonstrated that stroke victims can be trained to use an arm rendered useless by a stroke (Liepert et al., 2000; Taub et al., 1993). They accomplish this by restraining the normal arm and forcing the patient, through small increments of difficulty (a process known as shaping), to employ the affected limb for various tasks until it becomes useful once more, a technique learned from laborious work with deafferented monkeys (Taub et al., 1994). This new method is called Constraint-Induced Movement Therapy (CI Therapy). "CI Therapy changes the contingencies of reinforcement (provides opportunities for reinforcement of use of the more-affected arm and aversive consequences for its non-use by constraining the less-affected arm) so that the non-use of the more-affected arm learned in the acute and early sub-acute periods is counter-conditioned or lifted. Second, the consequent increase in more-affected arm use, involving sustained and repeated practice of functional arm movements, induces expansion of the contralateral cortical area controlling movement of the more-affected arm and recruitment of new ipsilateral areas. This use-dependent reorganization may serve as the neural basis for the permanent increase in use of the affected arm" (Taub et al., 1999, p. 241). This work has revolutionized the field of rehabilitation medicine.

PAIN

Animal research has revealed that specific pathways in the brain powerfully inhibit intense pain; that receptors in these same pathways bind morphine; and that the brain has its own opiate-like neurotransmitters, called endorphins, that function in these pathways (Basbaum and Fields, 1984; Mansour et al., 1995). More recently, scientists have identified molecules that regulate the endorphins (Mitchell et al., 2000). Targeting these molecules with selective antagonists may reduce the tolerance and some of the side effects typically associated with the use of morphine for pain control. Furthermore, research with awake, behaving animals found that stimulation of tiny electrodes that were implanted along pain-inhibiting pathways activated those pathways and effectively inhibited pain. With surgically implanted electrodes, some patients are able to press a button on a portable radio transmitter, activate the pain-

inhibiting pathways, and secure considerable periods of relief (Young et al., 1984). Relief has also been achieved for a different, much more frequently encountered group of pain patients, in whom the physical cause of the pain cannot be determined. This includes many patients with longstanding back pain. Treatments using principles of reinforcement and extinction, originally derived from experiments on animals, have eliminated these patients' dependence on narcotics and have restored many to normal activities (Fordyce et al., 1973; Roberts and Reinhardt, 1980).

Recent developments in the area of pain research use animal models of persistent pain that mimic inflammatory and neuropathic pain conditions in humans. In these conditions, stimuli that normally are not painful are perceived as painful. The severe pain that an arthritic patient experiences when fingers are moved is just one example. The animal models of these conditions have contributed greatly to our understanding of chronic pain and the development of new methods for controlling chronic pain (Casey and Dubner, 1989; Walker et al., 1999). Of great interest is a new appreciation that persistent pain conditions are not just a prolongation of acute pain processing, but rather result from changes in properties of the nervous system. These changes, which include the induction of new genes and the synthesis of new molecules, enhance pain processing, such that signals that normally are not painful become painful and persist (Basbaum and Woolf, 1999). Current development of pharmacological agents directed at the molecules that underlie these chronic pain-induced changes should significantly improve the treatment of pain in the near future.

PSYCHOTHERAPY

Previous to work by Dollard and Miller (1950), the psychosocial treatment of choice for non-psychotic disturbances consisted primarily of psychoanalysis practiced almost exclusively by medical professionals (McHugh, 2000). Dollard and Miller (1950) used the principles of learning derived from animal experiments as well as animal work on fear and displacement behavior to demonstrate that neuroses are learned and that psychotherapy could be considered a process in which the individual learns more adaptive social and emotional habits. The perception of psychotherapy as a learning process, following scientifically established principles of conditioning, positive and negative reinforcement, extinction, and so on, made its practice more accessible, both to practitioner and to prospective patient. More psychologists, as well as medical doctors thereafter, undertook the practice of psychotherapy. Today, practice is extended to various other help professionals, thus extending the supply of practitioners to meet the growing demand for services by an ever-broadening patient population.

Wolpe (1958) introduced a new therapeutic technique, systematic desensitization, based on the principles of learning theory. This technique used principles of reinforcement, counter conditioning, experimental extinction, and stimulus generalization derived from experiments

on animals. At the same time, students of Skinner began applying principles of behavioral analysis to human behavior problems. The coming together of these two streams of work resulted in the major development called Behavior Therapy that is now considered the treatment of choice for phobias, compulsions, and other neuroses, such as anorexia nervosa, that can produce misery and even death.

BIOFEEDBACK

Lubar (1987) has observed: "Biofeedback is a field that belongs to no one discipline. Although it developed from the principles of operant conditioning, which lie at the heart of experimental psychology, it is a field that is employed by virtually all health care disciplines and spans such diverse areas as dentistry, internal medicine, physical therapy and rehabilitation medicine, psychology and psychiatry, and virtually all the subspecialties of internal medicine."

Experiments with animals on classical and operant conditioning of visceral responses contributed significantly to the development of biofeedback (Kimmel, 1967; White and Tursky, 1982). Work has shown that humans can learn to control brain waves (Kamiya, 1969). Humans have also been shown to control the firing of single motor units—that is, a motor neuron and all the muscle fibers it innervates (Basmajian, 1963). These findings were based on earlier physiological experiments that discovered the existence of single motor units by studying the electrical activity of nerves in animals.

Evidence for the effectiveness of biofeedback has been well documented in the treatment of neuromuscular disorders, headaches, Raynaud's disease, orthostatic hypotension, hypertension, and fecal incontinence (Miller, 1985). The wide application of biofeedback techniques to treat incontinence in institutionalized elderly could save the United States as much as $13 billion a year (Rodin, 1984).

STRESS

The relationship between stress and its adverse medical consequences has a long history in both basic and clinical research. Experiments with animals, in which the confounding factors of research with humans can be rigorously controlled, have confirmed, for example, that psychosocial distress can contribute to the development of coronary artery disease. Social disruption and isolation have been shown to promote atherosclerosis in birds, swine, and cynomolgus monkeys (Ratcliffe and Cronin, 1958; Ratcliffe et al., 1969; Shively et al., 1989), through mechanisms involving hypothalamo-pituitary-adrenal axis and autonomic nervous system activation (Rozanski et al., 1999). Work in monkeys has been particularly important in demonstrating that personality traits along the dominance/subordinate spectrum can interact with environmental stress to influence the course of atherogenesis (Kaplan et al., 1982).

In the same way that animal models of *chronic* stress have contributed substantially to an understanding of the pathophysiology of coronary artery disease, the direct relation between *acute* stress and cardiac arrhythmias has been shown in dogs (Verrier, 1987). It is sympathetically mediated (Rozanski et al., 1999). That acute stress can also cause coronary artery endothelial damage has been demonstrated in rats, rabbits, and monkeys; these observations may be found to pertain to psychological factors operative during myocardial infarction in humans (Rozanski et al., 1999).

Animal models have played an important role in establishing that psychological stress can work together with *Helicobacter pylori* infection, or through independent pathways, to produce peptic ulcer disease (Levenstein et al., 1999). How genetic predisposition may modify the ulcerogenic potential of stress has been shown in studies of rat strains that differ as measured by emotional reactivity (Redei et al., 1994). Therefore, with increasing knowledge of the rat genome, insights at the molecular level into the neurobehavioral mechanisms underlying ulcer formation should be forthcoming. Other studies in rats are helping to identify the types of life experiences, and presumably associated psychological states, that modulate ulcerogenesis in response to a subsequent physical challenge (Overmier and Murison, 2000); these may have direct relevance to the design of preventive interventions in humans.

Animal models incorporating psychosocial distress occupy no less important a role in investigations of human mental disorders, as compared with medical disorders. The observation that "learned helplessness" could be induced in dogs and other species (Peterson et al., 1993; Seligman, 1975) served as one cornerstone of a widely held view that cognitive factors operate in precipitating and sustaining human depression (Willner, 1985). While a series of clinical studies has demonstrated the important role of psychological stress in the pathophysiology of the mood disorders (Kendler et al., 1992; McCauley et al., 1997; Roy, 1985), experiments in animals subjected to analogous stressors have offered insights into the underlying neurophysiological mechanisms. For example, work in rats has shown that excessive activity of corticotropin-releasing hormone (CRH) circuitry "may be the persisting neurobiological consequence of stress early in development" (Heim et al., 2000). Elevated CRHergic function has been implicated in many of the signs and symptoms of human depression (Nemeroff et al., 1984). The widespread use of the Porsolt swim test (by which immobility is induced in rats placed in a water bath) in screening and identifying anti-depressant drugs also attests to the importance of stress induction procedures in animals for understanding the mechanisms of human depression and its treatment (Porsolt et al., 1978).

Fear conditioning in animals involves forming an association between a neutral stimulus, discrete or contextual, and an aversive stimulus, generally a foot shock. The physiological consequences of fear conditioning strongly resemble human anxiety states (Davis, 1992), and a conditioned component to emotional responses has long been recognized in anxiety

disorders including posttraumatic stress disorder (PTSD) (Pitman et al., 1993). Therefore, conditioning procedures incorporating unconditioned stressors have occupied an important place in the study of anxiety. The neurocircuitry of the fear-potentiated startle response has been identified through an elegant series of investigations in rats (Davis, 1992); the continued application of pharmacological techniques to this model will almost certainly facilitate the design of new treatments for human anxiety disorders.

EFFECTS OF EARLY EXPERIENCE

Experiments on animals have confirmed, refined, and extended clinical observations on the long-lasting effects of infant experience. The demonstration of prolonged physiological as well as behavioral effects has motivated many significant efforts to enhance the beneficial and deter the detrimental effects of early childhood experiences (Hunt, 1961).

Investigators (Riesen, 1975; Wiesel and Hubel, 1965) have shown that various forms of visual deprivation cause permanent deficits in the development of visual connections in the brain. As a result of this work, pediatricians pay far more attention to the very early detection and correction of visual defects in infants, thereby reducing the occurrence of irreversible defects in adult vision (Moses, 1975).

Experimental studies with animals have also been key in demonstrating how the effects of early experience may be reversible. For example, Rosenzweig (1984) found that enriching the normally impoverished environment of infant rats produced more complex and elaborated play as well as the development of thick cortical brain layers. These thickened layers contained many more neural connections than those found in infant rats reared in an impoverished environment. These differences were discernible in adulthood. Enrichment works even in aged animals (Diamond and Connor, 1982) and can even reverse the effects of a genetic defect. Knockout mice lacking a receptor for an excitatory neurotransmitter in the hippocampus had many deficits in hippocampal-dependent cognition, yet environmental enrichment in these animals as adults overcame these deficits (Rampon et al., 2000).

Some infants that experience psychosocial deprivation fail to thrive and in extreme cases even become dwarfs. Brief periods of separation of newborn rats from the mother cause deficiencies in growth hormone and receptor function. The critical social deficit was not only the mother's absence, but also a lack of physical contact with the mother, especially a lack of the "stroking" that infant rat pups receive when the mother licks them. Stroking with a paintbrush can prevent or reverse both the hormonal deficits and the inhibition of growth (Schanberg et al., 1984). This knowledge has been directly applied to the clinical treatment of premature human infants. The aseptic conditions of incubators and nurseries for premature infants approximate maternal deprivation, evidenced by a disproportionate number of these infants failing to thrive.

DEFICITS IN LEARNING AND MEMORY THAT OCCUR WITH AGING

Experimental work with animals has had unique advantages in studying fundamental biological processes affecting cognitive behavior during the latter stages of aging. Because many animals age much more rapidly than humans (e.g., rats age approximately 30 times as fast as humans) experimental work with laboratory animals has enabled researchers to perform studies that would take decades or generations to conduct if limited to human subjects.

Experimental studies on a number of different species of aged laboratory animals have shown similarities in learning and memory to the learning and memory of aged humans (e.g., Bachevalier et al., 1991; Presty et al., 1987). Evidence continues to accrue that learning and memory acquisition (short-term memory) requires circuits through the hippocampus. Memory storage probably involves appropriate areas of association cortex (long-term store), and the retrieval and ability to manipulate data drawn from long-term storage (e.g., working memory) probably also requires intact circuits through the frontal lobe. Studies have more precisely identified the roles of the hippocampal and medial temporal lobe structures in the encoding and acquisition of new information and problems of memory with age. Recent findings indicate that stimulation of hippocampal neurons may result in proteins produced through the activation of immediate-early gene expression, which bind to specific synaptic phosphoproteins to consolidate the memory (Scanziani et al., 1996). In addition, transgenic models and mutant or conditional knockout mice with deletions, such as alpha-CAMKII and CREB (Silva et al., 1996; Kirkwood et al., 1997), may open windows to the underlying molecular mechanisms of age-related cognitive deficits, especially when linked to identification of such genes that manifest their effects late in life. These data could then be used in human population studies to determine the genetic linkages associated with behavioral and cognitive functions in the aging nervous system.

Research now indicates that generalized neuron loss leading to cognitive loss is not an inevitable consequence of aging. While there is an association between loss of cognitive function and thinning of cortical layer 1 and demyelination (Peters et al., 1996), aged monkeys appear not to lose neurons uniformly in the neocortex and hippocampus. However, studies in rats show that neuron number is preserved in aged animals and that degeneration of these cells and reduction in receptor sites are not associated with behavioral impairments (Rapp and Gallagher, 1996). Problems in memory are often observed in older adults, but research on the neural basis for these behaviors needs animal models to further our understanding of how to deal with these age-associated deficits. Work has been progressing in using transgenic animals and molecular probes to elucidate molecular mechanisms underlying learning processes and retention of memory. Animal models thus provide a powerful means for analyzing the neuronal mechanisms of memory deficits that occur with aging.

SLEEP DISORDERS

The recognition of rapid eye movement (REM) sleep in the 1950s (Dement, 1994) created an outpouring of research in cats and rats, in particular, that led to the development of a new branch of clinical medicine devoted to the diagnosis and treatment of sleep disorders 20 years later. The research on animals has greatly advanced understanding of the neural mechanisms underlying this extraordinary behavior in which the brain activity resembles that of alert wakefulness while the body musculature is paralyzed. Efforts to understand the latter ultimately led to the recognition and successful treatment of REM Behavior Disorder, in which the paralysis is overcome and people act out their dreams, which often results in serious bodily harm (Morrison, 1996).

The sleep disorder narcolepsy involves a disturbance of motor control and afflicts 0.05 percent of the population in the United States. Patients suffer from continual sleepiness and a strong tendency to experience partial to complete paralysis of their skeletal muscles while awake when presented with various emotion-laden stimuli or situations. There is no adequate treatment to relieve their misery. Genetic studies using dogs with a naturally occurring form of this disease, in which the sleep behavior has been studied for many years, and with mice have led to a recent breakthrough of identifying specific genes. These genes helped point researchers to a small collection of neurons utilizing peptides known as hypocretins in the hypothalamus. The connections of these neurons with other neurons long implicated in the regulation of sleep and wakefulness suggested that defects in their functioning could lead to various symptoms of narcolepsy, such as excessive sleepiness and cataplexy (Kilduff and Peyron, 2000). These studies led to the examination of the brains of narcoleptics, with the exciting result that very significant loss of the hypocretin neurons was found (Peyron et al., 2000; Thannickal et al., 2000). This was the first demonstration of a specific anatomical defect in this disorder. These findings are the first step in the development of targeted drugs that could help relieve the debilitating symptoms associated with the disorder.

In addition to specific sleep disorders, sleep loss, for a variety of reasons (many of which are linked to the hectic pace of modern life), can have a severe impact on human health and productivity (Kilduff and Kushida, 1999). Basic research on the mechanisms and genetics of circadian and homeostatic control of sleep may lead to a more complete understanding of the causes and effects of sleep loss. For instance, research encompassing a wide range of life forms, including bacteria, yeast, fruit flies, rodents, and humans (Dunlop, 1999; Johnson and Golden, 1999), has shed light on topics ranging from plant growth to understanding sleep patterns in animals and humans, which, in turn, has helped us better understand jet lag, shift work, and drowsy driving (Moore-Ede et al., 1982). ■

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CHAPTER 3

General Considerations

This chapter summarizes overarching issues that apply to all of the specialized topics that follow.

ROLE OF TRAINING, MONITORING, EVALUATIONS, TRACK RECORD

As indicated in the introduction, scientists work at the edge of what is known and cannot fully predict the consequences of any given manipulation. An immediate implication of this inability to predict consequences is the critical role of periodic training, ongoing monitoring, evaluation, and track record for animal care and use. New procedures are necessary for science, but they also need to be monitored and evaluated so that negative outcomes can be quickly corrected. The track record of individual investigators is an important indicator of future performance. An investigator experienced with an unusual species is often a leading expert on the care and welfare of that species.

OBSERVATION OF THE EXPERIMENTAL ANIMALS

There is no substitute for the regular observation of animals by both researchers and animal-care staff as well as a clear mechanism for reporting abnormal observations. Observational findings can be used to reduce experimental variance and errors by detecting adverse effects, unexpected illness, errors in food or water delivery, or equipment malfunction. One aspect of obtaining stable baseline performance is to have the same person conduct the experimental session from day to day (and to have consistency in the person who serves as backup). Animals serving in behavioral experiments are observed and/or handled one or more times daily by an individual familiar with the animal. As a result, an animal often becomes relatively docile around the person it is familiar with. Concomitantly, this person becomes very familiar with the animal's normal behavior and is able to readily discern changes. In addition to regular informal or systematic visual observation of the animal's behavior on a daily basis, routine controls are placed on such variables as amount of food (and sometimes water) consumed, so that changes in intake can be readily noted. Frequency of observations should be adjusted according to the speed at which an animal can be compromised in the experimental situation. Ideally, records should be readily accessible to veterinarians and staff with a legitimate need to see them.

TEAM APPROACH TO SETTING LIMITS

Laboratory animals, like humans, vary in their response to experimental conditions. When experimental conditions have potential consequences that may result in morbidity or mortality, the investigator, veterinarian (including animal care staff), and IACUC should work together to determine the appropriate limits beyond which the animal is removed or relieved of the condition(s) causing the morbidity. While the IACUC is responsible for approving protocols and the attending veterinarian can terminate experiments under certain conditions, the behavioral investigator is often in the best position to understand the risks for a particular animal in any experimental design and to detect animal pain or suffering in the course of an experiment. To the extent possible, it is valuable for the investigator to anticipate and define limits and endpoints in protocol preparation and review stage. It is in the interest of the animals and the institution for the IACUC, the veterinarian, the animal care staff, and the investigator to work together as a team to foster good animal care and good science.

LEVEL EVALUATION OF THE EXPERIMENTAL VARIABLE

It is difficult to make general conclusions from a study that uses only one level of an experimental variable (e.g., drug dose, stimulus intensity, or reinforcement magnitude). The results of an experiment are influenced by many variables. In an effort to maintain the consistency of their data, researchers may reduce the number of variables in their experiment. However, it is wise to keep in mind that results may not be similar if obtained under a different combination of variables. For this reason, "recommended" values for an experimental variable (e.g., the number of hours of fluid restriction, the number of amperes of electric shock) are not provided in this document. Experience has taught that the critical value of certain parameters may change substantially depending upon other variables (e.g., the animal's species, age, sex, and history of exposure to the experimental variable).

SPECIES OF ANIMALS

This document addresses methods proven for use with rodents, the species used in much of the research and teaching in the United States. Considerable attention also is devoted to methods with nonhuman primates to gain insight into welfare issues, because they are important models in behavioral studies. Behavioral research methods similar to those reviewed here have also been used to study large farm animals (e.g., Arave et al., 1992). Investigators using farm species should consult the National Research Council (NRC) Reports for those species (ILAR, 1996; Federation of Animal Science Societies, 1999). Chapter 9 of this report, Ethological Approaches, reviews procedures for studies of behavior in the wild, which often involves species not traditionally used in the laboratory.

STRESS VERSUS DISTRESS

For scientific investigations, stress is an elusive concept, with almost as many different definitions as there are investigators. At the core of most definitions, however, is the notion

that stress is a departure from physiological and behavioral homeostasis with the "stress response" resulting in behavioral and physiological adaptations designed to return the organism to homeostasis. This definition includes stressors that are not harmful and may be beneficial—for instance, gravitational stress is necessary for maintenance of bone density. The prevalent thinking is that stress becomes harmful when it is sufficiently prolonged or is of such a magnitude that adaptation is not successful or not possible. Thus, a distinction is often made between the inability to adapt and a stressor. Understanding this distinction from a scientific perspective is the topic of intensive ongoing research.

ROLE OF ADAPTATION, HABITUATION, AND CONDITIONING

The state of adaptation, habituation, or conditioning for any organism is an important consideration in determining the acceptability of any proposed treatment or experimental condition. The aversiveness and harm of procedures such as restraint, drugs, and other stressors are highly dependent on the history and experience of the animal. For example, cold conditions that may be entirely normal or even important for wild animals may be unacceptable for unconditioned laboratory animals. This also means that all individuals responsible for the care and use of animals must be appropriately trained on the natural biology and proper laboratory handling of the species under study.

IMPORTANCE OF SPECIES AND ETHOLOGICAL CONSIDERATIONS

Animal welfare rules have been designed around the species and preparations most common in laboratory practice. It is up to the IACUC to judge the appropriateness of such rules for the species and experimental conditions in a given protocol; deviations to the regulations must be scientifically justified, and animal welfare must be optimized given the experimental conditions. Nevertheless, some exemptions require waivers from the USDA. The IACUC has been given wide latitude to provide exceptions to the rules where it is required by needs of a particular species. Thus, some species may be harmed by a continuous flow of fresh air in ethological laboratory settings or by the stainless steel environment of the typical animal care facility. Under such circumstances, with an appropriately written rationale, the IACUC should consider a deviation from standard laboratory animal practice.

CHANGE IN ETHICS, VALUES, AND KNOWLEDGE

The principal investigator, the IACUC, and the animal care staff must be aware that they are working in an environment in which there are ongoing changes in scientific knowledge and public values, which in turn will require regular re-evaluation of protocols. Strong, ongoing communication between the IACUC, the veterinarian, the animal care staff, and the investigator is essential to managing these changes smoothly.

PROVIDE OCCUPATIONAL HEALTH SERVICES

Two NRC Reports including *Occupational Health and Safety in the Care and Use of Research*

Animals (1997), are excellent resources providing guidance for the protection of those who use animals in research (ILAR 1996,1997). It is essential to have an occupational health and safety program based on the identification of hazards and the reduction of risks. Risk assessment plays an important role in an effective occupational safety and health program. Unprotected exposure to animals carrying infectious agents can have potentially negative and possibly fatal consequences for researchers and staff—for example, caretaker deaths caused by cercopithecine herpesvirus 1 (CHV 1) transmitted by macaques and hantavirus transmitted by rodents. Allergies also pose substantial health risks to sensitized persons. Although the essential elements of an occupational health and safety program will vary across species, common factors include vaccination history, protective clothing, and training of all personnel contacting the animals. Because animals in behavioral studies generally are not anesthetized, management practices must protect the health and safety of both animals and staff. Handling methods that provide the most freedom to the animal without compromising the restraint objective or personnel safety are desirable. For example, the risk of bites or injury to the handler may be reduced by using transfer boxes rather than by relying on direct handling of the animals. Additional references to handling methods can be found in Chapter 5, Experimental Enclosures and Physical Restraint. ■

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CHAPTER 4

Manipulation of Food and Fluid Access

Delivery of food or fluids is commonly used to maintain extended sequences of behavior in studies with a wide range of animals. Species as diverse as dolphins, goats, pigs, sheep, cows, turtles, fish, octopuses, and crabs, as well as the more often used rats, mice, pigeons, and monkeys, have been trained to perform simple to complex tasks under training procedures in which small amounts of a food or fluid (referred to as rewards or reinforcers) are used to maintain performance.

REGULATED VERSUS FREE ACCESS TO FOOD AND FLUIDS

The widespread use of food or fluid reinforcers is due to their well-studied ability to motivate the development of a new behavior and to maintain stable responding for extended periods. Many experiments require weeks or months of experimental sessions (five to seven days per week), and require that stable performance be maintained from day to day. Experimental sessions can be very short (e.g., 10 minutes) or long (e.g., 12 hours); some studies conduct sessions intermittently or continuously over 24 hours (e.g., time course of drug effects).

Control of access to food or fluid outside the experimental session ensures response reliably to the food or fluid reinforcer in each session. Maintaining performance reliably, even with a "treat," is better done in food-restricted animals than those fed *ad libitum*. There are additional reasons to control access to food. Many behavioral experiments seek to maintain weights within a constant, narrowly defined range, because fluctuating weights and/or hours of food restriction can be potential sources of behavioral variability. When animals have free access to food, the amount eaten in the hours just before experimental testing may vary. Also, weight regulation per se may be important as one means of minimizing other sources of variability in experimental results. In drug studies, for example, control of the animal's weight, and in some cases the spacing of meals, helps ensure uniformity of dosing across time.

Restricted food access (either in laboratories or in the wild) is not unusual or undesirable. Experiments have demonstrated that a number of species are healthier and live longer if they

are not allowed to become obese (Ator, 1991; Kemnitz et al., 1989, 1993; Lane et al., 1992, 1997; Turturro et al., 1999). For example, rats having dietary restriction sufficient to cause a 25 percent reduction in body weight compared to controls fed *ad libitum* lived longer without impairment of growth or of routine clinical indices of health (Hubert et al., 2000). Weight restriction is best started after the animal has reached maturity. Problems occur only if the ration is nutritionally incomplete or unbalanced.

Restriction of caloric intake, in the context of ensuring a nutritionally balanced diet, is recognized in the 1996 ILAR Report (ILAR, 1996) as an accepted practice in long-term housing of some species. In the wild, food and water generally are not "freely" available; that is, effort (foraging) is required to obtain them. Ethological observations indicate that most species have access to food and water only for limited periods of each day (Altman and Altman, 1970; Hall, 1965; Hamilton et al., 1976; Lindburg, 1977). Thus, research methods that require animals to expend time and energy to obtain food during limited periods each day can be compatible with the natural pattern. In fact, USDA regulations permit "task-oriented" access to the regular food supply as a means of environmental enrichment for laboratory primates.

'TREATS' VERSUS BALANCED DIET AS FOOD REWARDS

Although "preferred" food items or "treats" often are used to maintain stable responding, balanced pelleted or liquid diets have several advantages over treats, such as sugar pellets or sweetened condensed milk. It is important to note that the nutritional status of the animal may be better if the majority of calories are obtained from balanced diet rather than treats (i.e., even if balanced diet is freely available, animals may eat less of it if they receive a significant number of calories from treats). The possibility of dental caries with frequent consumption of sugared food is also a disadvantage, particularly when the subjects will serve for many months or years.

SPECIES DIFFERENCES IN WEIGHT REGULATION

The manner in which food restriction is accomplished and any target weight selected must be carefully considered for the species in question to maintain the animals in good health and to adhere to humane standards of care. The reduced weight often seen as a "generic" standard in the literature for a variety of species is 80 to 85 percent of a free-feeding weight. The age of the subject and the duration that free feeding is permitted, however, are critical determinants of whether the "80 percent" rule is a reasonable one for different species. Knowledge of nutrient requirements as well as feeding and growth patterns for different species is important to determine rational weight control regimens. The goal is to select a weight range that permits the reinforcer to maintain responding during the experimental session and maintains the animal's physical well-being. Another factor to consider is that a lower weight may be necessary early in training but not after performance has been established, even though food

control will still be needed. Information on a few commonly used species is summarized below (see Ator, 1991, for references and additional coverage).

With rats, it is especially important to consider the age of the rat and the duration of free access to food at which the 100 percent weight was determined if reduction to a percentage of that weight is to be used. Rats of some strains (e.g., Sprague-Dawley, Long-Evans hooded) are semi-continuous feeders and can gain weight almost indefinitely. In such rats, waiting for weight to stabilize in order to determine a free-feeding weight is not practical. If rats attain a relatively high weight (e.g., 500 grams), 80 percent of that weight may not be a weight at which training will occur rapidly. On the other hand, if a free-feeding weight for a young rat is quite low (e.g., 200 grams), 80 percent of that weight maintained over the rat's life span may be unnecessarily restrictive (Heiderstadt et al., 2000). The best restricted-weight criterion is one at which the rats work reliably for food reinforcers, remain healthy, and live as long as possible (i.e., two to three years) in studies in which sacrifice is not an experimental endpoint.

The weights of mice tend to reach an asymptote relatively quickly, but strains differ considerably. Weights should be permitted to rise to a reasonably stable maximum under free-feeding conditions before they are decreased by restricted feeding. Although stable reduced weights can be maintained easily in mice, accidentally missing a day of feeding may prove fatal, in contrast to such regimens with other species.

Free-feeding guinea pigs steadily gain weight for 12 to 15 months before weight asymptotes. Use of food or water reinforcers can be problematic. Some investigators found that restriction of either had deleterious effects, but success with particular edible reinforcers (e.g., carrot juice, sucrose solutions, a milk and cereal mixture, and commercial guinea pig pellets) has been described for guinea pigs maintained under restricted feeding.

Pigeons tend to self-regulate feeding under free-access conditions, and stabilization of the body weight of an adult bird occurs in two to four weeks. The 80 percent body weight regimen is most easily used in this species. A typical procedure is to weigh the bird after the session to determine the amount of supplemental feeding. The bird is fed the difference (in grams) between the current weight and the target weight; with experience, investigators often are able to determine an additional amount that can be fed such that the bird will be at, rather than below, the target weight for the next experimental session.

With nonhuman primates, the rate of metabolism and the rate of growth can vary significantly even within the same species. Food restriction (e.g., one individualized post-session feeding per day), rather than reduction to a specific target weight, usually results in stable behavioral baselines. Types of reinforcement used with nonhuman primates vary greatly. The one chosen is governed by a complex interaction involving the research question,

requirements of the experimental apparatus, length of the experimental session, length of the experiment, and cost. Restriction to some percentage of a free-feeding weight may be necessary for initial training or for study of certain experimental questions, but the particular percentage necessary may vary across individual monkeys. Nonhuman primate species differ in their nutrient and energy (gross kilocalorie per kilogram of body weight) requirements. Familiarity with requirements for the species is important if food restriction is to be used, particularly if feeding will consist primarily of food pellets formulated for use as reinforcers for monkeys. Some species may need a vitamin supplement. Nonhuman primates require a dietary source of vitamin C; providing a supplement of fresh fruit or vegetables daily or a couple of times a week helps prevent vitamin C deficiency and also serves as a means of environmental enrichment.

GENERAL PROCEDURES AND CONSIDERATIONS

Unless specific protocols require exemption, allowing most laboratory animal species to feed at least once per day is consistent with standards of humane care, and is required for species covered by USDA regulations (see review of research by Toth and Gardiner, 2000). Information on the daily caloric, nutrient, and water requirements of many species is published in the ILAR Report, *Nutrient Requirements of Domestic Animals Series* (ILAR, 1995). Balanced animal diets, which consider these recommendations, are available commercially as pellets for reinforcement for a variety of species. As long as the expiration dates are heeded, the diet is all that is needed to feed laboratory animals appropriately under free-feeding conditions. Under restricted feeding conditions, however, vitamin supplements may be used, depending on the species. Supplements also may be appropriate when feeding is not particularly restricted but amount consumed is likely to decrease as a function of some experimental manipulations, such as surgical interventions or administration of some drugs.

Constant access to water typically is provided under food control regimens. There is interdependency between food and water intake (e.g., food-restricted animals may drink less water), but species differ in their patterns of drinking during the day and in their response to food restriction.

Food-restricted animals typically are weighed frequently, usually before experimental sessions. Species whose weights change slowly under minimal restriction regimens may be weighed less often if some form of anesthesia (e.g., ketamine) is required to accomplish this. However, animals on food restriction must have their body weight recorded on a regular schedule.

Once animals are trained under many behavioral procedures, they may continue to serve as subjects over their life spans. A factor to consider is whether there will be a return to unrestricted food in periods between studies. Practices vary and there are several considerations. These include (1) the extent to which weight was restricted below an *ad*

libitum weight during the study; (2) the probability that a new *ad libitum* weight is desirable because of the age of the animal at the time of original determination (or because of seasonal variations in weight with adult male squirrel monkeys); (3) the extent to which particular species tend to "waste" or scatter food (e.g., monkeys) under free-feeding; and (4) whether there are problems created by abrupt shifts between restricted and unrestricted feeding (e.g., bloat in some monkeys).

REGULATING ACCESS TO FLUID

When water is used to maintain stable responding, access to water outside the experimental session needs to be controlled. The influence of varying amounts of water restriction on operant performance has been described (Hughes et al., 1994). In addition, some other liquid reinforcers (e.g., fruit juice with monkeys) under certain conditions (e.g., procedures that require long sessions with many reinforcer deliveries) may also maintain performance most reliably when access to water is controlled.

Fluids have advantages over solid food reinforcers for behavioral procedures that might require that the animal's head be kept in a particular position (e.g., psychophysical studies or studies that monitor brain activity in awake, behaving organisms). In such cases, the fluid may be delivered through a solenoid-operated sipper tube positioned at the animal's mouth. A particular advantage of fluids when an experiment involves neuronal recordings with microelectrodes is that chewing or crunching movements of the teeth or jaws does not occur when the animal is consuming the reward.

Animals physiologically tolerate a lack of food better than a lack of water. Determining parameters of water restriction that do not produce dehydration or excessive weight loss requires careful consideration. Animals need not be at risk if intervals of fluid access and total amounts of fluid obtained are appropriate to the species (ILAR, 1995; Toth and Gardiner, 2000).

Some studies using fluid delivery to maintain a behavioral performance require that the animal earn its daily fluid requirement during the experimental session, and these sessions typically are multiple hours in length. Other studies use shorter sessions, but provide a period of supplemental access to water shortly after the session. On days when sessions are not conducted, animals should receive a period of access to water, unless there is strong experimental justification for not doing so (e.g., when duration of fluid restriction is an independent variable).

The main disadvantage of fluid control in very small animals is the risk of rapid dehydration if the animal fails to receive its daily water requirement. A good system of daily monitoring procedures is essential under such protocols. Records should be kept of the amount of fluid

earned in the task as well as any supplements given. Careful observation of the animal's behavior and regular clinical monitoring of the animal's health are critical for ensuring successful application of fluid control procedures.

Body weights should be monitored several times weekly. Animals under water control may lose weight over time due to reduced food consumption. Food should be given in close temporal proximity to the access to fluid (e.g., immediately after the session). Monitoring the amount of food consumed daily is a quick way to determine if adequate fluid intake is occurring. A plan of action should be in place in advance and implemented in case weights decline to unhealthy levels under a fluid control regimen.

REGULATING THE TASTE AND CHEMICAL COMPOSITION OF FOOD AND FLUIDS

Experiments may require manipulation of food or fluid intake in order to study hunger, thirst, taste, and olfactory senses. Methods for these experiments have been summarized (Wellman and Hoebel, 1997). For example, a two-choice preference test would offer the animal two containers, one with plain food or fluid, the other with a test substance added (Cunningham and Niehus, 1997). Special diets should be evaluated for spoilage and degradation. Record-keeping is critical. Pre-printed forms help to ensure consistent recording of the lot number of each diet, the amount consumed, body weight, and notes about the animal's appearance, equipment problems, departures from the protocol, and so on. Methods for presenting drugs and other experimental chemicals in the food and water are discussed in Chapter 6, Pharmacological Studies.

A FINAL NOTE ON FOOD AND FLUID CONTROL

When beginning work with a new species, consult with the laboratory animal veterinarian as well as recent literature for that species before designing protocols that require restriction of food or water. When the study begins, be prepared to consider and address a range of behavioral, environmental, or equipment-related variables that might hinder training or disrupt performance. Inexperienced personnel may presume that a source of problems in training or maintaining a food- or fluid-motivated behavior is that the restriction is not strict enough (or, in some cases, that it is too strict). The other types of variables that should be considered first, however, are equipment malfunctions, programming errors, task criteria that are raised rapidly or set too high for the animal's level of training, illness, or nonprogrammed water restriction (in the case of food-motivated behavior). In all circumstances, careful monitoring of animals under food or fluid control is necessary every day to avoid additional nonprogrammed restriction. ■

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CHAPTER 5

Experimental Enclosures and Physical Restraint

TYPES OF APPARATUS

Most behavioral experiments involve transferring the animal to a specially constructed apparatus, such as an operant chamber ("Skinner box"). There is a long tradition of studying the behavior of rodents in various kinds of mazes (including a water maze), running wheels, or open field areas (Porsolt et al., 1993). Whatever specialized chamber is used, the animal remains in it for the duration of the experimental session, and then is returned to the home cage. Such apparatus is usually interfaced to a computer and equipped for presentation of stimuli (e.g., lights, sounds, food pellets) and to record behavior (e.g., lever operation, licking a spout, locomotor activity). Depending on the experiment, the apparatus into which the animal is placed may or may not be placed inside a larger chamber that is designed to attenuate extraneous visual or auditory stimuli during the experimental session. Ator (1991) reviewed the use of chambers and other apparatus.

Some behavioral experiments require restriction of movements during the experimental session. For example, restraint is commonly used in cognitive or neurophysiological experiments that use awake, behaving monkeys to study sensory function, perception, learning, and memory. In such experiments, it is important to ensure a consistent orientation toward and precise distance from sensory stimuli. In those cases, a specially designed sling or chair may be used. Head restraint may be used if it is important that the animal (usually a monkey) look at a fixation point on a video monitor so that eye position can be monitored and/or if activity of the central nervous system (e.g., electrical activity of brain cells) is being monitored during the behavioral task. Often the chair itself will incorporate devices (levers, lights, feeders) needed during the experimental session. In other situations, the chair is wheeled in front of an intelligence panel.

In other types of behavioral experiments, the animal's activity may be restrained by means of a tether. For example, in intravenous drug self-injection experiments or ones that require intra-gastric drug delivery, the animal (e.g., rat, mouse, monkey, dog) may have been implanted with a chronic indwelling intravenous or intragastric catheter (e.g., Lukas et al.,

1982; Lukas and Moreton, 1979; Meisch and Lemaire, 1993). The catheter is arranged to exit from a site on the back (typical in monkeys) or the top of the head (typical in rats and cats). Then the catheter is threaded through a protective device, referred to as a tether, and the tether is connected to a swivel. The tubing emerges from the swivel and is connected to a pump, which is used to deliver the drug. Monkeys that have been fitted with chronic indwelling catheters often wear specially designed vests, shirts, or harnesses to protect the catheter exit site. Special procedures (e.g., using antiseptic or aseptic precautions when connecting the end of the catheter to the swivel) are carefully planned to maintain the animal in good health and maximize the life of the catheter.

Experiments that require presentation of electrical stimuli to the brain or recording changes in sleep and wakefulness involve equipping the animal with a chronic indwelling centrally implanted electrode. Some experiments require one or more chronically indwelling cannulae in a ventricle or other specific region of the brain (e.g., those involving central drug injection or *in vivo* microdialysis) (Barrett, 1991; Goeders and Smith, 1987). Typically, connection to the tether or tubing is made at the beginning of the experimental session and removed at the end when the animal is returned to the home cage.

When experimental conditions must remain in effect for 24 hours at a time, animals with chronically indwelling catheters live in the experimental chamber, or the home cage is equipped with an intelligence panel to permit presentation of stimuli and recording of responses.

CONSIDERATIONS

Many forms of restraint and many different kinds of experiments are acceptable as long as the particular procedures for inducing and monitoring restraint are well justified, minimized as much as possible, and consistent with the ILAR Report (ILAR, 1996). Sometimes the behavior of interest is exploration of a novel environment (e.g., open field activity measures in rodents). In other cases, exposure to restraint may be an independent variable in an experiment (e.g., to take physiological measures believed to be affected by unfamiliar restraint). In many of the cases described above, however, a habituation phase is carried out before the experiment itself begins. Because animals in behavioral experiments are handled frequently (often five or even seven days a week), they usually become habituated quickly to the procedures of transfer to the experimental apparatus or chair and to procedures of attaching and removing tethers.

The habituation phase is especially important for experiments that will involve the greater restriction on movement. For example, habituation of a monkey to a shirt/harness/tether arrangement is best carried out well in advance of the planned date for implantation of the catheter. Inspection of the animal periodically during this habituation process allows the experimenter to determine if the vest fits well and permits adjustments to prevent chafing.

For experiments using chairs, one can train macaques and squirrel monkeys to move voluntarily from the home cage into a chair that is used during the session (Ator, 1991). In one common method, monkeys wear a collar with a small metal ring attached. The monkeys come to accept having a chain clipped to the collar, which then is pulled through a ring at the top of a metal pole. Squirrel monkeys usually grasp the pole and ride to the chair on it, while larger monkeys, such as adult macaques, learn to walk to the chair. By holding that end of the pole snugly at the collar and pulling the chain down to the end of the pole, the experimenter can control the monkey's movements and be protected from the possibility of a bite in the process of training and transfer. Larger monkeys can be trained to move from the home cage into a smaller shuttle device that can be wheeled to the experimental chamber. Treats are used during the various steps of training the monkey in the transfer process and during habituation to sitting in a chair. The amount of time the monkey is actually seated in a chair or remains in an experimental chamber might be gradually extended during training. The monkey should not live in the chair, though.

Just as with jacket or harness devices, animals that are restrained in a chair must be monitored to ensure that chafing or bruising does not occur. If ulceration or bruising should occur, the monkey should be removed from the study until the area is healed, and adjustments should be made to correct the source of the problem. As long as the investigator monitors the animal to ensure, among other criteria, that the restraint chair permits reasonable postural adjustment, does not interfere with respiration, and does not cause skin abrasions, this form of restraint can be used safely. **The best evidence of behavioral adaptation to the restraint and tolerance to experimental conditions is voluntary movement into the device and performance of the behavioral task once there.** ■

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CHAPTER 6

Pharmacological Studies

The administration of a drug or toxicant to animals being observed for behavioral effects can be justified by the need to understand the chemical's role in causing health problems for humans or animals (e.g., drug dependence, neurotoxicity), or the need to understand whether the drug can alleviate health problems (e.g., pharmacotherapy for behavioral and neurological disorders). Some research is designed to characterize the behavioral effects of an unknown chemical (e.g., the assessment of the abuse liability of new pharmaceuticals). It also is important to determine whether an organism's response to a drug changes because of chronic exposure to it and whether such exposure may lead to abuse or physical dependence.

Another category of research examines chemicals that are known or are hypothesized to have specific behavioral effects that the investigator wishes to understand in more detail. For example, research with a drug commonly abused by humans is aimed at delineating the mechanisms underlying the drug's reinforcing or rewarding effects. Other research in this category examines how experiential and environmental variables influence the behavioral response to a drug.

Drugs can be used to illuminate physiological and/or neurochemical mechanisms of behavior. A drug that blocks a neurotransmitter receptor system can help to determine the neurotransmitter's role in producing a specific behavior. A drug may be administered because it can produce anxiety reactions so that the research may understand the biological and behavioral consequences of chronic stress and possibilities for therapy. More detailed information is provided in the several chapters and books on behavioral pharmacology and toxicology (Branch, 1993; Ellenberger, 1993; Goldberg and Stolerman, 1986; Meisch and Lemaire, 1993; Seiden and Dykstra, 1977; van Haaren, 1993; Weiss and O'Donoghue, 1994).

BEHAVIORAL BASELINES

In many behavioral experiments that include drug administration, the animals are trained to perform some response that can be objectively measured. The motivation for the response often is delivery of an appetitive or a drug reward (as in self-administration studies) or, less often, the avoidance or escape from some aversive condition (see Chapter 7, Aversive Stimuli). Trained responses usually involve operating a lever or switch. Other dependent variables are

feeding or drinking or some form of locomotor or exploratory activity (Iversen and Lattal, 1991; van Haaren, 1993; Wellman and Hoebel, 1997).

A critical element to many studies is the establishment of reliable and stable performance of the target behavior as a baseline against which to judge the drug effect. Especially when trained behaviors, such as lever pressing, are used, experimental sessions are conducted five to seven days per week. These may be brief (e.g., 30 minutes) or they may be long (e.g., three hours). In some experiments (e.g., those studying self-administration or drug dependence or the time-course of a drug effect), the experiment may run virtually continuously (24 hours/day).

In drug discrimination studies, animals are trained to make one response after receiving a dose of a drug and to make a different response after receiving saline (placebo). After repeated pairings, the internally perceived drug serves as a cue (technically termed a discriminative stimulus) that controls which response is made. Testing consists of sessions in which a novel drug is presented to the animal. Thus, the investigator can "ask" the animal to tell, by its differential response, whether or not it "feels" the drug.

CONSIDERATIONS RELATED TO HOUSING AND SOCIAL GROUPING

Exposure to drugs usually necessitates individual housing in order to permit repeated access to each animal for dosing and testing. Individual housing also may be preferred because, in a group situation, drug-altered behaviors may increase an animal's risk of abuse by cage mates, as well as impair its ability to compete for food. For animals in studies of intravenous drug self-administration or of constant intragastric infusion, the animal may be fitted with a vest and tether apparatus to protect the chronically indwelling cannula, as described in Chapter 5. Behavior may be measured in the animal's living cage, to which devices for presenting stimuli and recording responses have been attached (Ator, 1991; Evans, 1994). Such arrangements may preclude conventional group housing.

Behavioral experiments in pharmacology often employ restricted access to food or water for two purposes: (1) to maintain a consistent motivation of behavioral performance (Ator, 1991) and (2) to standardize content of the digestive tract for uniform absorption and uptake of orally administered drugs. This involves scheduling the availability of food and water but not necessarily deprivation. In addition, for experiments that take place over many weeks, it may be important to keep the total amount of drug delivered relatively constant, even when drug doses are calculated on a per weight basis.

PHARMACOLOGICAL VARIABLES

DOSE-EFFECT RELATIONSHIPS

A hallmark of behavioral pharmacology research is the determination of dose-effect relationships. That is, a range of doses is selected that encompasses one producing no or very little effect up to one at which the animals do not perform the target response. Dose-effect relationships may be determined by studying single doses in separate groups of animals (between-subject designs) or by determining a full dose-effect relationship in each animal (within-subject, or repeated-treatments designs). The baseline performance usually is reestablished between sessions in which a drug is given.

Drug doses given by the experimenter can be given acutely (e.g., a single injection of a drug before a session once or twice a week) or chronically (e.g., once or more daily for some length of time), but there is a range of variations. In drug interaction studies, two doses, each of a different drug, would be given at appropriate temporal intervals before the behavioral test. Cumulative dosing procedures may be used. In these, increasing doses of a drug are administered within a relatively short period, and a brief experimental session is conducted after each dose. The effects of the drug are assumed to cumulate in an additive manner so that within a period of two to three hours the effects of a range of doses can be determined (Wenger, 1980).

Drug self-administration experiments determine the drug's reinforcing efficacy, which may indicate the drug's potential for abuse. The animal controls the number and frequency of delivery of the test drug. That is, a quantity of a particular drug is available intravenously, orally, or via inhalation, and the subject of interest is the amount of behavior maintained by this drug at the self-administered dose. In such studies, the dose available is varied across experimental conditions, and the rate of responding to obtain the dose, the number of drug deliveries obtained, and/or the amount of drug taken are the primary dependent variables of interest. In such studies, the likelihood that the animal will produce a fatal overdose is carefully considered in the design and choice of drug. Drugs vary across classes in how likely it is that high drug doses will produce adverse effects. Overdose may be minimized by placing an upper limit on the number of doses per session or on the minimum time-lapse between doses, or by setting the magnitude of each dose available to the animal.

DRUG VEHICLES

Most drugs are provided to researchers in solid form and must be dissolved or suspended in a liquid carrier (vehicle) in order to be administered. Aqueous vehicles (e.g., sterile water, saline) have no pharmacological action of their own; however, many drugs need more complex vehicles (e.g., one that has an organic solvent, such as propylene glycol, or an alcohol). Testing with the vehicle, without a drug, will provide a control for the vehicle's

influence on performance as well as a determination of any effects of the drug administration procedure itself. Where animals serve as their own controls, they typically become habituated to the dosing procedure, and behavior is not different from that in sessions not preceded by dosing. The exception to this may be if a vehicle or vehicle/drug combination irritates the tissue into which it is injected (e.g., due to high or low pH). Lesions can be eliminated or minimized by using less concentrated solutions or alternating injection sites. If less concentrated solutions require volumes that are too large for single injection sites, delivery may be made by small volume injections at different sites. In some cases, one can adjust the pH by adding another chemical after the drug is dissolved (although the solubility limitations of some drugs preclude much adjustment).

ROUTE OF ADMINISTRATION

In many cases, the rationale for choosing a route of administration will be dictated by goals of the study (including comparability of results with previous studies); in other studies, it may be dictated by constraints on the solubility of the drug. In many studies, more than one route is compatible with the goals of the research; the route may be chosen according to factors such as the route used with humans, the animal species, and/or information about the metabolism of the compound.

The routes of drug administration used in studies with animals have included oral (*per os*, p.o.), subcutaneous (s.c.), intramuscular (i.m.), intraperitoneal (i.p.), intragastric (i.g.), intravenous (i.v.), inhalational, or intracranial (e.g., into the ventricles or to a specific brain region). Some routes are more practical for some species than others, and an important variable is precision of the amount of drug the animal receives. Drugs can be given orally by gavage needle (e.g., rats, pigeons) or nasogastric tube (monkeys). Injection by hypodermic needle is the most frequently used technique for administering drugs and chemicals in behavioral research (Iversen and Iversen, 1981; van Haaren, 1993). The site of injection may be determined by the characteristics of a particular drug's absorption or the solvent in which it is given. The most likely problems are incorrect site of injection during i.p. injection. These problems can be minimized by careful training of personnel and by prior adaptation of animals to the handling and restraint that normally accompany injection. The frequent handling of animals in behavioral studies by the same individual usually results in an animal that is quite well habituated to regular injection procedures.

Direct insertion of a cannula, temporarily or chronically, into a blood vessel, a body cavity (e.g., the stomach), the spinal cord, or the brain is another route of drug administration. A permanently implanted cannula ensures that repeated injections can be given at precisely the same site and permits the study of drug effects without peripheral effects (e.g., pain at injection site). Implantable pumps for slow delivery of a drug also are used for chronic drug exposure studies, such as studies of the effects of drug tolerance or physical dependence on

behavior (Tyle, 1988). Aseptic technique is important in the implantation of cannulae or pumps and whenever the system must be opened (e.g., to reattach tubing or add drug solution). These precautions will greatly reduce morbidity in the animal and prolong the useful life of the cannula.

Inhalation is the most common route of exposure for some agents (e.g., nitrous oxide and organic solvents or anesthetics). Administration of some compounds is simplified as with nasal sprays, but usually inhalation exposures require specialized experimental chambers or equipment to control drug exposure and to protect laboratory personnel and other animals from accidental exposure to the airborne chemical (Paule et al., 1992; Taylor and Evans, 1985). The risk of hypoxia requires attention when drugs are administered by inhalation for long durations. Questions of drug abuse by smoking can be modeled with animals (e.g., Carroll et al., 1990).

Studies in which animals are provided the opportunity to self-administer a drug often employ the i.v. route, and the animal will be implanted with a chronically indwelling venous cannula. Cannulae are common in self-injection studies with rats, monkeys, dogs, and mice (e.g., Lukas et al., 1982). They generally are guided subdermally from the implantation site to exit in the midscapular region and protected by a vest (see Chapter 4, Experimental Enclosures and Physical Restraint). They may remain chronically attached to the infusion system or be attached only when the animal is moved to the experimental chamber. Methods for intraventricular drug self-administration through cannulae implanted directly into the brain also have been developed (Goeders and Smith, 1987). Several drug self-administration procedures that use the oral route also have been developed (Meisch and Lemaire, 1993). They may employ a specialized drinking spout to regulate the volume of each drink (often termed a drinkometer). In these studies, access to a regular supply of drinking water typically is not restricted or is restricted only during the experimental session itself so that the drug reinforcing efficacy can be determined in the absence of fluid restriction. Choice of route of drug delivery for self-administration studies is complexly determined by the purposes of the experiment and the nature of the drug and its pharmacokinetics, just to mention the most prominent variables.

To study the effects of chronically administered drugs or toxicants, oral delivery may be accomplished by adding the compound to the animal's food or drinking water, as in some models of alcoholism (Cunningham and Niehus, 1997) and studies of long-term exposure to toxic contaminants of food and water (Cory-Slechta, 1994). Special feeders and water canisters (Evans et al., 1986) are available to prevent spillage. When a drug is added to food or water, it is important to monitor the animal's ingestion, both for determining the amount of drug received and to identify reduced ingestion resulting from reduced palatability. If

consumption of the food is reduced, it is wise to include a pair-fed control group to determine whether results are attributable to the drug or to the reduced caloric or fluid intake.

HEALTH CONSIDERATIONS

Behavioral pharmacology experiments generally are designed to avoid irreversible effects or potential loss of the animal. Some behavioral toxicology experiments, however, will involve dosing that produces cumulative deleterious effects. A contingency plan that addresses the conditions under which side effects are to be alleviated or the animal is to be removed from the experiment should be planned for in the protocol.

DRUG SIDE EFFECT

Some drugs studied in behavioral pharmacology, particularly when dosing is frequent, will affect feeding and drinking, activity level, and other bodily functions (e.g., elimination). Nevertheless it can be too easy to assume that alterations in such processes are an effect of the drug and thus to overlook other causes of behavioral changes during a drug study (e.g., dental problems that affect food consumption).

PHYSICAL DEPENDENCE

Although mere repeated administration of a drug will not necessarily produce physical dependence on a drug, physical dependence can sometimes occur as a consequence of repeated dosing procedures (Goldberg and Stolerman, 1986). A characteristic withdrawal syndrome upon cessation of the chronic dosing regimen reveals physical dependence. The features of the withdrawal syndrome and the rapidity with which it appears after the drug has been stopped are idiosyncratic to the nature of the drug that has been chronically delivered (e.g., the opioid withdrawal syndrome differs from the barbiturate withdrawal syndrome). The severity of the withdrawal syndrome typically is an interactive function of the daily dose and duration of the period of chronic drug delivery. In addition, individual animals, particularly from outbred strains, will differ somewhat in the particular signs and symptoms they exhibit in withdrawal and in the apparent degree of severity. Some experiments involve deliberately administering a compound under a particular regimen in order to study physical dependence to the drug; however, where the dosing regimen is one in which it is known that a withdrawal syndrome could occur, it is reasonable to anticipate the possibility and suggest steps that could be taken to diminish discomfort in the protocol.

Whether or not there is treatment of a withdrawal syndrome in the laboratory depends on the purpose of the experiment and the nature of the withdrawal. If the purpose is to study the nature of the withdrawal syndrome, including whether or not there will be such a syndrome (e.g., for newly developed compounds), then providing pharmacological treatment to ameliorate it may be antithetical to the purposes of the experiment. It is always desirable, though, to have a "contingency plan" for treatment if a life-threatening sign occurs (e.g.,

seizure). In cases in which feeding and drinking decline to some predetermined level, it is important to have a contingency plan for alternative feeding. Withdrawal is, by definition, a time-limited phenomenon, and thus true withdrawal signs revert toward a pre-drug baseline level over time after drug withdrawal. If the withdrawal syndrome is not a subject of study, dose-tapering regimens or substitution of other drugs to ameliorate withdrawal can be implemented for drugs for which it is known that the withdrawal syndrome can be severe after prolonged administration (e.g., opioids, barbiturates), just as they would be with humans. In cases in which the withdrawal syndrome is very brief and/or mild, however, dose tapering is not necessary.

DURATION OF DRUG OR TOXICANT EXPOSURE

In experiments involving study of the direct effects of chronic exposure (e.g., possible deterioration of performance under repeated exposure to a neurotoxin or the development of tolerance to an initial effect of a drug), two questions require particular attention: the length of drug exposure and the disposition of the animal. The decision to end chronic drug exposure typically is based on predetermined criteria that establish a range of changes from baseline behavior that will be considered significant. Termination of exposure may also be planned to obtain tissue specimens. The observation of overt signs of toxicity, however, may necessitate a decision to terminate treatment earlier than anticipated. Daily observation of animals by someone familiar with the experimental protocol is especially important in studies involving chronic drug or toxicant administration so that timely decision-making can occur.

LONG-LASTING DRUG EFFECTS

The dosing regimens used in many behavioral studies do not produce long-term effects or behavioral impairment. After an appropriate wash-out time, the researcher can determine the existence of long-lasting or irreversible effects (Bushnell et al., 1991). Irreversible effects are not a problem if the protocol calls for the animal to be sacrificed to obtain cellular data to supplement the behavioral results. Another factor in the decision to sacrifice is when it is believed that chronic drug exposure altered a physiological or behavioral function that compromises the animal for use in future studies. On the other hand, such an animal would be a valuable resource when the aim of the research is to understand mechanisms of tolerance, post-exposure recovery, or therapeutic interventions that ameliorate long-lasting drug effects. ■

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CHAPTER 7

Aversive Stimuli

Aversive stimuli (technically termed negative reinforcers) are, by definition, those that an organism will avoid or escape. One can evaluate empirically whether a particular stimulus (e.g., an electric shock, a loud noise, a cold environment) will serve as a negative reinforcer by presenting it and determining whether a laboratory animal will learn a response that prevents it, terminates it, diminishes its intensity, or decreases its frequency of occurrence. Stimuli that function as negative reinforcers for some individual species are not aversive for others. The same is true, of course, for positive reinforcers. As with positive reinforcers, however, it has been determined that some stimuli will function reliably as negative reinforcers across a wide range of conditions for most organisms. Electric shock is such a stimulus, which partially accounts for the prevalence of its use as an aversive stimulus in behavioral research. Other aversive stimuli might be critical in some areas of research, such as studies of pain.

Behavioral studies that use aversive stimuli fall into several broad categories. There are those that examine aversively motivated instrumental behavior, such as avoidance, escape, and punished responding. Classical fear conditioning is one of the most commonly used behavioral paradigms in which aversive stimuli are employed. In fear conditioning, the aversive stimulus, usually footshock, is paired with some neutral event, and as a result the neutral stimulus acquires the ability to elicit emotional behaviors and physiological adjustments that typically occur in the presence of stimuli that cause harm or predict danger. Because these responses are hard-wired, they result in species-typical expressions. Fear conditioning is often said to be stimulus rather than response learning (i.e., the means by which humans and other animals learn about novel dangers). Other researchers focus on pain, while some study aversive conditions commonly referred to as "stress."

AVERSIVELY MOTIVATED BEHAVIOR

Many different stimuli have been used to study aversively motivated behavior, such as deviations from ambient temperature (Carlisle and Stock, 1993; Gordon et al., 1998), a puff of air under pressure (Berger and Thompson, 1978; Welsh et al., 1998), a novel cage or an unfamiliar animal (Gould et al., 1998; Miczek, 1979; Miczek and O'Donnell, 1978; Weninger et al., 1999), strong visual or auditory stimuli (Crofton, 1992), restraint, and electric shock (Honig, 1966). Systematic manipulation of an aversive stimulus permits the establishment of

a variety of behavioral baselines from which to select the one best suited for the experimental question.

The basic behavioral paradigms of aversively motivated instrumental (or operant) behavior are escape and avoidance. An escape procedure is one in which an animal learns to make a particular response to terminate contact with an aversive stimulus that is already present (e.g., electric shock through a grid floor that can be escaped by running to another compartment of the apparatus or by pressing a lever that turns the shock off). An avoidance procedure is one in which an animal learns that making a certain response will prevent an encounter with an aversive stimulus. For example, in one passive avoidance procedure, a rat learns not to step off a platform due to experience with shock delivered through the floor below. In a common type of active avoidance procedure, an animal learns that steadily operating a lever will prevent shocks from occurring (unsignaled avoidance) or that pressing a lever when it hears a particular tone or sees a particular light will prevent a shock from occurring (signaled avoidance).

Another behavioral paradigm is a punishment (sometimes termed conflict) procedure (Azrin and Holz, 1966). In this procedure, making a response occasionally produces a positive reinforcer (e.g., food of some sort); but some or all of the responses also produce an aversive stimulus, which has the effect of reducing the overall rate of responding maintained by the food. Different degrees of suppression can be produced by varying parameters such as intensity of the aversive stimulus, or the number of responses followed by the aversive stimulus.

Extensive research on paradigms that use negative reinforcers revealed much about the behavioral processes that operate under such conditions (Azrin and Holz, 1966; Baron, 1991; Campbell and Church, 1969; Morse et al., 1977). Consequently, researchers who wish to establish reliable baselines of aversively motivated behavior to examine the effect of other variables (e.g., the effects of psychoactive drugs or of the modulation of particular neurotransmitters) can rely on that literature to determine experimental parameters that are most suitable.

In studies of avoidance or punished behavior, once the animal acquires the response, it is common for few if any shocks to be delivered (i.e., the delivery is under the animal's control). The experimental focus in these studies is on the reliable performance of the response itself and the effects of experimental variables that will alter the probability of this response.

The behavioral paradigms described above typically use lever operation as the response. Other types of behavioral research require aversive conditions but study different behaviors. An aversively motivated paradigm that is important in research on the neurobiology of depression and in research on antidepressant drugs is a forced swim test, used in rats (Lucki, 1997;

Porsolt et al., 1978). Some studies use drug administration to create a noxious effect (e.g., nausea by lithium chloride) to study phenomena such as the development of conditioned aversions (e.g., avoidance of an otherwise palatable solution that had been paired with lithium chloride) or to study the effects of drugs on conditioned aversions. In the conditioned suppression paradigm, an unavoidable aversive stimulus (usually electric shock) is signaled by a distinctive sound or light; the animal learns to suppress ongoing behavior, typically responding for food, in the presence of that stimulus.

ELECTRIC SHOCK

Electric shock is by far the most frequently used aversive stimulus in research. Although a number of other aversive stimuli have been used in a variety of studies, there are characteristics of electric shock that have made it particularly useful as an aversive stimulus in a variety of laboratory research. An electric shock stimulus, whether applied through a grid floor or a carefully placed electrode, has several advantages from an experimental and humane perspective.

In the range used for behavioral research, electric shocks do not produce tissue damage. Shock produces its noxious quality by directly stimulating nociceptive fibers rather than by producing injury. The sensation produced by electric shock does not persist beyond the period of stimulation, and the stimulus does not interfere with the ability to respond (e.g., under a punishment or conflict procedure). It is interesting to note that researchers who test the shock levels on themselves report that it is not clear whether shock in the intensity range typically used causes "pain" in the traditional sense, or if the sensation produced is more accurately described as a very unpleasant sensation.

Physical aspects of the shock stimulus are specifiable and controllable by the experimenter, which has advantages for the subjects as well as for the experimental design. The type of shock, voltage, current, duration, number of shocks, and body area to which shock is applied all can be precisely stated and thus precisely controlled and replicated within and across laboratories. An extensive literature on shock parameters (Azrin and Holz, 1966) minimizes the amount of exploratory work needed for selecting stimulus parameters before the actual experiment.

STRESS RESEARCH

Stress research has as its purpose the production of an objectively determined stressful state in order to study various behavioral and physiological sequelae. For example, the research may investigate the behavioral and/or physiological changes involved in animal models of depression. Not all research that uses aversive stimuli seeks to produce stress per se, and it is an unresolved empirical issue whether objectively determined stressful states are necessarily present under all aversively motivated paradigms. An example is whether an animal that

serves in an avoidance procedure manifests objective indices of stress under conditions in which responding is so efficient as to avoid any shock deliveries. The development of reliable, objective indices of stress is important to stress research (i.e., those are the dependent variables in many studies). At the same time, information from such studies can also inform our understanding of the effects of other behavioral procedures that use aversive stimuli.

Events that will serve as stressors are quite specific to species, systems, and processes, and thus different stressors are used for different purposes. For example, in examining the effects of stressors on immune function, there are several important considerations. Many of the dysfunctional processes that are typically associated with stress have been found to occur only if stress is relatively severe or prolonged. For example, depletion of norepinephrine in the locus coeruleus occurs only after exposure to intense stress, and increases in serum cholesterol are produced after exposure to repeated stressful sessions but not after a single session of stress. Studies of stress, then, must employ lengthier exposures to aversive stimuli than would occur in studies in which the primary goal is to develop behavior motivated by a negative reinforcer.

In stress research, subjects often do not have control of the aversive stimulus. Many of the phenomena that are most relevant for human health occur only, or most readily, if the subject does not have control. Control is a form of coping, and the deleterious effects of exposure to stressors are most evident when coping is not possible. Therefore, to add the element of coping or control to a study on the deleterious effects of stress could be inconsistent with the goals of the study.

No single physiological or behavioral measure can be taken as uniquely indicating the occurrence of stress response. Certain behavioral changes, if persistent, often are assumed as evidence of stress. These are decreases in grooming, ingestion, body weight, locomotor activity, exploration, aggression, or sexual behavior. Increased "freezing" is also considered to be indicative of stress. Although this list indicates some assessments that can be made to determine the existence and degree of stress, some indicators may not be useful in all situations. Further, these signs are not exclusive to aversive stimuli or to stressful environments. For instance, decreased food intake and reduced body weight are concomitants of illness. The relationship between aversive stimuli and the behavioral, physiological, and hormonal changes is a topic of ongoing research. Although corticosterone concentration in blood is sometimes regarded as a physiological indication of stress, no index is uniformly accepted as a more reliable indicator of "stress" than behavioral evidence.

PAIN RESEARCH

Just as many studies of aversively motivated behavior do not seek to investigate stress, many of those studies do not seek to investigate pain, although it is presumed that the pain of a

stimulus such as electric shock provides the motivating condition to learn an avoidance response. However, some behavioral studies are concerned with pain per se.

Those researchers studying pain have recognized and addressed ethical issues surrounding this type of research. Guidelines for pain research in animals were developed early on by the International Association for the Study of Pain (Zimmermann, 1986) and have been updated by the American Association for Laboratory Animal Science (AALAS, 2000).

Animals should be free of pain except at times when the experiment will be compromised by avoiding or eliminating it. Whether pain is a by-product of a research procedure or a focus of study, certain principles remain the same. In the latter case, the animals should be exposed to the minimal intensity and duration of pain necessary to carry out the experiment. A consensus on the application of this principle turns out to be much more difficult to achieve than one would think. For example, the intensity of an aversive stimulus that is suitable for motivating avoidance behavior may not be an intensity that is suitable for a study of stress on immune function or for study of analgesia.

A committee of the International Association for the Study of Pain has defined pain in people as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Anonymous, 1979). Animals cannot give a verbal description of the pain, but pain can be inferred from physiological and behavioral changes, because animals exhibit the same motor behaviors and physiological responses as people in response to painful stimulation. These responses include withdrawal reflexes, vocalization, and learned behaviors such as pressing a bar to avoid further exposure to an aversive stimulus or to decrease its intensity.

Principles developed for experimental studies of pain in humans should be applied in pain research on animals. Human subjects are exposed only to painful stimuli that they can tolerate, and they are able to remove a painful stimulus at any time (see the discussion of chronic pain below). Tolerance for pain needs to be clearly distinguished from the threshold for detecting a painful stimulus. It is when the intensity of the stimulus approaches or exceeds the tolerance threshold that our behavior is dominated by attempts to avoid or escape the stimulation. When the animal cannot control the stimulus intensity, it is critical that the experimenter determine the level of pain produced by stimuli. Although controllability of the aversive stimulus is often consistent with achieving the goals of the research in studies on pain, it might be inimicable to study of stress.

PAIN ASSESSMENT METHODS

Scales for rating clinical manifestation of animal pain have not proven to be very reliable (Flecknell, 1996). Thus, objective behavioral measures are employed in animal studies on

pain. Latency measures often are used to assess reflex responses. For example, in the tail-flick reflex, a radiant heat stimulus is focused on the tail and the animal flicks its tail to escape the stimulus. The effectiveness of analgesic agents in this model is highly correlated with their effectiveness in relieving pain in humans. More recently, the tail-flick reflex has been used to assess pain produced by brain stimulation, stress, or the microinjection of opioids. Other reflex measures include the flinch-jump and the limb-withdrawal tests in which mechanical stimulation produces a brisk motor act. Behavioral reflexes in amphibians can be used to evaluate analgesics (Stevens, 1996). These simple reflex measures have limitations, but they all permit the animal to have control over stimulus magnitude and thus ensure that the animal can control the level of pain to which it is exposed. The tail-flick reflex has the added advantage of being functional under light anesthesia.

More complex, organized, but unlearned behaviors are often used as measures of pain because they involve a purposeful act requiring supra-spinal sensory processing. A commonly used method is the hot-plate test in which a rat or mouse is placed on a plate preheated to 50° to 55°C. A paw-licking response is measured. A method has also been devised in which rats receive heat stimuli through a glass plate while they stand unrestrained in an experimental cage (Hargreaves et al., 1988). The rats withdraw their limb reflexively but also exhibit complex behaviors, such as paw licking and guarded behavior of the limb. A latency measure and the withdrawal duration (how long the limb remains off the glass plate) are used to infer pain. All of the above methods provide the animal with control of the intensity or duration of the stimulus because the motor behavior results in removal of the aversive stimulus.

A variant of an escape procedure that has been useful in studies of analgesia is the shock titration procedure, in which the animal operates a lever to decrease the intensity of electric shock (Dykstra et al., 1993). Failing to press the lever results in increases in the intensity, which can then be driven down again by lever operation. In this manner, shock intensity thresholds can be determined. The most common and simplest escape paradigm involves the animal's escaping an aversive stimulus by initiating a learned behavior such as crossing a barrier or pressing a bar. The latency of escape is usually used as a measure of pain experienced. Other more complex methods include reaction time experiments in which the animal signals the detection of an aversive stimulus by operating a lever.

Learned behaviors have an advantage over simpler, unlearned behaviors in that the magnitude of the behavioral change varies with the stimulus intensity, thus providing reliable evidence that a change in behavior reflects the perception of a noxious stimulus rather than merely a change in motor performance. Sophisticated behavioral tasks in animals also allow the experimenter to rule out changes in performance that are related to attentional and motivational variables rather than changes in pain perception (Dubner, 1994).

CHRONIC PAIN MODELS

The past decade has seen the proliferation of animal models to study the effects of tissue and nerve injury on the development of persistent or chronic pain. In most of these studies, the animals are awake and perceive pain. These models attempt to mimic human clinical conditions. A major purpose of such studies is to further knowledge that can ultimately be applied to the management of acute and chronic pain in humans and animals. There is a special need to demonstrate responsibility in the proper treatment of animals that participate in these experiments. The animals should be exposed to the minimal pain necessary to carry out the experiment. Models of inflammation that may produce more persistent pain include the injection of carrageenan or Complete Freund's adjuvant into the foot pad (Dubner, 1994). These models result in persistent pain that mimics the time course of postoperative pain or other types of persistent injury. Studies have shown that the impact of the inflamed limb on the rat's behavior is minimal and the rats will use the limb for support if necessary. Recently developed models indicate that partial nerve injury in the rat results in signs of hyperalgesia and spontaneous pain and mimic neuropathic pain conditions (Dubner, 1994). These neuropathic pain models have been adapted to mice recently for studies of transgenic animals. All of the inflammation and nerve injury models that attempt to mimic human pain conditions produce pain that the animal cannot control. Therefore, it is important that investigators assess the level of pain in these animals and provide analgesic agents when it does not interfere with the purpose of the experiment. Pain in these studies can be inferred from ongoing behavioral variables such as feeding and drinking, sleep-waking cycle, grooming, guarding of the limb, and social behavior. Major changes in such behaviors may indicate that the animal is in considerable pain and the experiment should be terminated.

OTHER CONSIDERATIONS

Although the concept of using minimal levels of intensity of shock, as with any stressor, is an important one, research has shown that higher intensities or numbers of shock sometimes need to be used in certain types of studies. First, in stress research, the effect of reduced movement can be achieved after 40 inescapable shocks; interference with learning begins to occur after 80 shocks but is clearer after 120 shocks (Minor et al, 1988). Second, research on punishment has shown that using gradually increasing shock intensities results in habituation. That is, the level of shock ultimately required to produce the desired suppression of responding will likely be higher than if a higher shock level had been used initially. Because there is considerable adaptation to shock if it continues for many sessions or if it is given in chronic form, shock may have disadvantages for long-term stressor experiments unless adaptation per se is under study. Third, the same shock applied to the same body region sequentially activates different neural pathways that regulate pain as the number of shocks increase.

It is almost universally assumed that controllable and predictable aversive events are preferable to unpredictable and uncontrollable stimulation. Careful psychophysical study has revealed, however, that predictable shocks are perceived as more severe or intense than unpredictable shocks, and there are conditions in which controllable shocks are more stressful than uncontrollable shocks. Indeed, in many studies using shocks that are not under the subject's control, the shock durations are much briefer than those that are under the subject's control. One often uses shock durations of 0.5 to 1.0 second in classical conditioning studies. But, a behavioral response that requires moving from one location to another may require several seconds for the subject to terminate the shock.

In certain studies, control over the stimulus entails a tradeoff for subject and investigator. If disturbances in catecholamine metabolism are the object of study, these disturbances come into play only when the aversive stimulus is of a specified intensity and uncontrollable. If controllable shock is used, the shock intensity required to produce measurable effects would be much greater than the intensity required by uncontrollable shocks.

The effect of any given shock stimulus varies according to a wide range of variables: history of the subject, species used, waveform of the voltage, body region shocked, size of the electrode or diameter of grids, and series resistance. For example, shock stimuli that produce vigorous reactions in the rat are often undetected by pigeons. If electrodes are used, current density increases as the size of the electrodes decreases; if grids are used, current density varies as the animal moves across grids, with current density increasing as grid size decreases. Experienced investigators select shock parameters by taking account of the complexity inherent in these and other variables.

CONCLUSION

Past research on aversively motivated behavior and stress has yielded data that can inform researchers in designing studies that use aversive stimuli (see References). Each experimental procedure that uses aversive stimuli has its own set of technical methods, advantages, disadvantages, and cautions. In addition, methodological details of a given stressor or aversive stimulus differ according to the species of animal used as subjects. Investigators should make clear the reasons that a specific procedure is most appropriate for a given study, the advantages and disadvantages of the procedure, and the impact of the procedure on the organism under investigation. ■

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CHAPTER 8

Social Variables

Social factors come into play in behavioral research in two ways: (1) research directed at study of the influence of social variables upon behavior and (2) the behavioral consequences of husbandry techniques. Investigation of social variables in animal subjects can be used to help understand human problems (e.g., separation and loss). Manipulation of social variables (e.g., individual housing) may be necessary for performance of other research. Both will be addressed below.

SOCIAL VARIABLES AS RESEARCH TOPICS

The individual and societal cost of atypical human behavior indicates the importance of research with animal models of social problems. Social behavior in many species, including humans, may be based in large part on social attachment, a special type of relationship involving recognition of and response to the individual, rather than the conspecific organism. First seen in the mother-infant relationship, social attachment in humans extends to peer-peer relationships, perhaps even to non-animate relationships, and may serve a psychobiological regulatory function. Paradigms involving alterations of early developmental experience can be used for investigation of the manner in which altered early social experience contributes to the development of individual, social, and parenting behavior, and for studies of the basic neurobiological mechanisms underlying such behaviors and behavioral pathologies.

POPULATION DENSITY

Manipulating the number of animals housed in a limited physical environment is one means of investigating the behavioral and biological effects of social stimuli. In a variety of species, high-density housing leads to prolonged changes in cardiovascular and immune functioning. Given these known effects on health and well-being, high density should be used only when adequately justified by research goals and should not be employed as a routine or long-term condition. Guidelines for housing density are shown in Tables 2.1, 2.2, and 2.3 of the ILAR Report (ILAR, 1996).

GROUP FORMATION AND INTRUDER PARADIGMS

Behavioral research can involve the study of the formation of new social relationships or the effects of introduction of a new individual into an established social group or territory. When humanely employed, these procedures have been effective in studying aggressive behavior and the behavioral responses to stress (Miczek, 1979; Miczek and O'Donnell, 1978). Evidence of serious wounds or an inability to maintain normal homeostatic functions should be used as criteria for terminating the research condition. Aggression may be the primary focus of the research (Boccia et al., 1989), may be a useful by-product (e.g., alpha animal using titrated aggression in the social control of other animals), or may be an unwanted by-product of social manipulation (e.g., in formation of primate social groups).

SOCIAL SEPARATION OR ISOLATION

While the formation of new social relationships is potentially stressful, the dissolution of established relationships can be equally important. Separation techniques are used to study the effects of loss, or disruption of social attachment bonds/relationships. These paradigms have served as animal models of depression, of the effects of social relationships on behavior and biology, and of long-term effects of early separation or loss experiences on later development.

Species that exhibit "aunting" behavior (sharing of infants by adults) may be associated with less marked infant responses to separation. Langurs, for example (Dolhinow, 1980), exhibit relatively little distress when separated from their natal mothers and adopted by other adult females within the group. Similarly, adult female bonnet macaques (M. radiata) will frequently share care of young infants, such that the infants develop close bonds with adult females in addition to the mother. When the mother is removed from the infant in these groups, and the infants remain in the social group with familiar adults with whom they have established a previous relationship, the separation response is muted both behaviorally and physiologically (Laudenslager et al., 1990; Reite et al., 1989). With rodents, methods for cross-fostering of pups are routinely used.

SOCIAL DEPRIVATION

Research involving prolonged social isolation, particularly of young animals, may be evaluated depending on whether the isolation is required as a specific focus of the research, a necessary corollary of the research protocol, or an inadvertent occurrence based on practical or husbandry considerations. Where separation or social isolation is the subject of the research, the justification of separation must draw upon the considerable knowledge that has been gained from this type of research. Manipulations of the early rearing environment of animals have provided important insights into the development of social and affective behaviors, as well as sensory functions. This area of research has also provided convincing support for the role of the parent in promoting normal cognitive and emotional development.

When social separation or isolation is proposed as a research manipulation, several issues should be considered. These include the species and age of the animal; its ability to maintain itself independently; the frequency and duration of the separations to be experienced; and the evaluation procedures used by the investigator. The future requirements of the animals should also be considered.

The oversight of research involving social factors is an especially difficult area of consideration for IACUCs for several reasons. Opinions differ on the social needs of various species. Definitions of terms such as "stress" and "well-being" are vague. And the task of balancing research goals against evolving standards of animal care is precarious. A key factor in any consideration of social variables is the known predilection of all organisms to adapt and cope with changing environmental conditions. Many investigators have documented changes in behavior that occur with changes in social or physical stimuli in the caged animal's environment (e.g., Evans et al., 1989; Hubrecht, 1995), but there are few instances in which the animal's new "behavioral budget" is clearly an advance in health outcome. Although this section emphasizes research methods, the influence of social factors in husbandry will be described briefly because these factors influence behavior and have become a standard component of husbandry practices for some species (ILAR, 1996, pp. 37–38). Bayne and Novak (1998) provide an excellent review of variables that influence behavioral pathology in captive nonhuman primates.

BEHAVIORAL IMPLICATIONS OF MANIPULATING SOCIAL VARIABLES

SOCIABILITY OF THE SPECIES

Early research suggested that some animals (many primates and rodents) may have an innate "gregarious" tendency that predisposes them toward social living, whereas others (adult male primates and some carnivores) are more inclined to live solitary lives. Human experience and further animal studies show, though, that the tendency for or against sociality is influenced by early rearing conditions. Group-rearing of rodents or macaques in infancy may foster a *preference* for social housing, whereas the same species may find social living aversive if derived from a less social rearing environment. The full extent to which "social needs" can be modified by the rearing environment remains an empirical question.

GROUP FORMATION AND INTRUDER PARADIGMS

Routine husbandry will at times require the formation of new social relationships, as individual animals are retired from the experiment and new animals replace them. Incompatible pairs or groups should be separated and more appropriate companions found, when available. When aggression is not the focus of the research, it is especially important in

the formation and changing of social group structure in primates to attend to aggressive interactions, to minimize the amount of antagonistic interactions, and to protect the health of the group members. It may be helpful to permit animals to become acquainted before they are placed in the same group—for example, housing them in proximity to each other, or placing a potential new group member into the social group in a smaller cage for a time before releasing it.

GENDER OF THE ANIMAL

Post-pubertal males of many species exhibit aggression toward other males, and for this reason they cannot be housed together.

AGE OF THE ANIMAL

The social needs of animals vary across the life span, even in gregarious species. Data exist for many species showing that appropriate social stimulation is important for normal infant development. Special consideration thus needs to be given to the normal parental rearing of infant animals, unless the focus of the research itself precludes this. At the other end of the life span there is evidence in some species (including some nonhuman primates) for a decline in sociality with old age. Thus, the recommendation for social companionship must be flexibly and appropriately applied.

TYPE OF SOCIAL PARTNER

To achieve the benefits of social companionship, thought must be given to the optimal type of social partner. Even in gregarious species, many competing behavioral processes influence the positive or negative nature of social relationships. The formation of hierarchical dominance relationships may affect the relative benefits of social housing for each individual. Subordinate animals, for example, may have more difficulty obtaining food or freely moving around in the spatial environment. This concern is most evident in newly formed social groups, where it can be expected that the influence of dominance will subside somewhat over time unless desired resources such as food or water are limited. It can also be assumed that the sex and age of the partner will influence the nature of social relationships that are formed, and thereby the relative benefits/costs of sociality for each individual. Data are needed to weigh the benefits to animal and researcher of social housing against negative consequences (disease transmission, aggression).

RESOURCE AVAILABILITY

When animals are housed socially, careful consideration must be devoted to the manner in which resources are provided. Food and water may have to be presented *ad libitum* to prevent competition for limited resources, or they may have to be presented in a dispersed manner, so there will be less competition for resources at a restricted site. The ideal environment would provide individuals with the opportunity to separate themselves from social companions while

feeding, but providing this may result in prohibitively large spatial and physical demands on the research environment.

SEPARATION FROM THE SOCIAL GROUP

Questions about social separation will become more common as more research subjects are socially housed. Negative impacts of these separations can be minimized. For example, the effect of social separation is aggravated by simultaneously placing animals in an unfamiliar environment, whereas allowing the animal to remain in the home cage after removal of the companion reduces the effects. Similarly, placing the infants with other familiar companions reduces the effect of weaning infants from the mother.

Extensive studies with nonhuman primates have indicated that the largest effects are observed in the first day after social separation, although some physiological changes may persist for one to two weeks. Both behavioral manifestations of distress and altered physiological responses return to normal after this time, and it is often difficult to distinguish the animal from its prior social baseline period by overt measures.

Separation of infant primates from each other at four to six months of age is associated with a pronounced behavioral protest reaction (Suomi et al., 1976), but the physiological manifestations and effects of separation are by no means as prominent as is the case for mother-infant separation (Boccia et al., 1989). Macaques separated from members of their nuclear family also exhibit behavioral protest reactions (Suomi et al., 1975), although the physiological correlates of such separations have yet to be identified.

Pair or group housing may be incompatible with some research protocols for some animal species. Individual housing may be necessary for animals receiving continual administration of experimental diets or drugs, experiments monitoring food and water intake, or experiments from which there is regular collection of biological samples. Individual housing may be necessary to prevent social companions from handling the research subject's implanted instrumentation or attacking the subject while it is recovering from drug treatment.

Potentially deleterious effects of individual housing can be minimized if carried out in an environment that permits visual, auditory, olfactory, and even limited tactile contact. Additionally, alternative stimulation and activities can be offered to such subjects during the period of restriction. Efforts should be made to minimize individual housing where possible in animals previously raised in social environments. Chronicity of the treatment and age of the subject should be evaluated in devising creative alternatives—for example, adjacently house two familiar subjects when instrumented or surgically implant the instruments in inaccessible locations. Emerging technologies may increase our ability for remote recording of experimental data, further limiting the requirement for individual housing. Physiological

monitoring can often be performed in social groups by means of totally implantable telemetric devices (Pauley and Reite, 1981), and implantable osmotic minipumps can be used to deliver pharmacological agents in animals living in social groups.

MOTHER-INFANT REARING

Macaque monkey infants raised exclusively with their mothers without additional social experience may exhibit species-typical social behaviors, but there is some evidence that such individuals may also exhibit excess or inappropriate aggressiveness (Mason, 1991; Woolverton et al., 1989). These behaviors may result from inadequate contingent social behavioral feedback and could also compromise the ability to extrapolate data from such subjects to socially reared individuals, and complicate breeding programs dependent upon these animals. Such infants can be removed from their mothers when they are able to feed on their own, although they will exhibit a separation reaction, with both behavioral and physiological components, if they are separated at much less than a year of age. They will generally be socially competent adults, although possibly exhibiting atypical aggressiveness.

SOCIAL MANIPULATIONS: EXPOSURE TO UNFAMILIAR ANIMALS

Much of the ethological literature is focused on the reactions of animals to members of their own or other species. This research runs the gamut from studies of breeding behavior or group formation to those that examine communication processes. Animals may be exposed to other conspecifics or to specific attributes of those conspecifics such as their odors or vocalizations. Welfare considerations will vary depending upon both the context and the extent of the exposure. For example, when the exposure occurs between two or more unfamiliar animals, care should be taken to minimize the risk of aggression and injury. In some cases, bringing unfamiliar animals together may require the use of introduction cages or other techniques to provide a period of familiarization under controlled conditions. For example, creating breeding pairs of some rodent species may require more effort than merely placing the animals in the same cage. To eliminate aggression, males can be placed in a small mesh introduction cage within the home cage of the female and then released several hours later (as appropriate for the species and individuals).

MIXED SPECIES INTERACTIONS

Occasionally different species may be housed together. Primates can be reared in mixed species environments for economic as well as for scientific reasons. The African savannah is a mixed species environment, as are many modern zoos. Compatibility of species is important, and mixed species offspring may occur, which may or may not be desirable. One of the more common procedures is to cross-foster young to the parents of a different species in an attempt to unravel genetic and environmental influences on behavior. This approach has been used to study the acquisition of song in birds, behavioral development in rodents, and patterns of aggression and reconciliation in monkeys. Several cautions should be noted in the cross-

fostering paradigm. First, the time of cross-fostering is generally critical to its success. For some species, fostering must occur within the first day or two of life (e.g., voles). When the timing is unknown, offspring should be monitored carefully for signs of rejection or neglect. Even when parents care for offspring, continued monitoring for signs of malnourishment may be necessary. Second, there may be significant health risks in housing certain species together. Finally, cross-fostering can lead to altered species-typical behavior in adulthood (e.g., in terms of mating preferences and patterns of parental care). The study of behavioral differences attributable to fosterers may be the focus of research, but cross-fostered animals may be unsuitable for *routine* use in breeding colonies because their offspring may differ substantially from the species norm.

SEPARATION FROM CONSPECIFICS DURING DEVELOPMENT

Some research involves separating animals from conspecifics during development. In some cases, the separation is necessary in order to provide the animal with alternative rearing environments (e.g., rearing nonhuman primates with inanimate surrogates and/or peers) or with controlled stimulation from conspecifics (e.g., use of playbacks in song acquisition in passerine birds). In other cases, the process of separation is of interest (e.g., mother-infant separation in nonhuman primates).

When animals are separated from parents through experimental protocol, the investigator and the animal care staff must assume responsibility for rearing the offspring. Adequate attention must be paid to the temporal provisioning of food, actual food intake, nutrition, warmth, and other biological needs. Consideration must also be given to the possible stress produced by the loss of companions. In this regard, both the timing and the type of separation may be crucial. Offspring that are separated at birth or shortly thereafter may not yet have formed strong social bonds with their parents and peers. In contrast, offspring separated later in development may show acute stress followed by depression in response to separation from conspecifics (e.g., three-month old rhesus monkey infants separated from their mother). The type of separation will also affect the response of the offspring. Separation in which an infant is removed from its social group and placed in a new environment by itself may be considerably different from separation in which a particular conspecific such as the mother is removed from the social group and the infant in question remains behind with the other group members. Regardless of the kind of separation, young animals should be monitored closely and evaluated regularly. Further, the long-term consequences of any developmental separation should be considered, and the long-term care of adversely affected animals should be addressed. The above discussion pertains to separation during early development and not to removal of juveniles following a natural weaning process, as is the practice of those caring for and maintaining rodent and other breeding colonies (Reite, 1987).

NONHUMAN PRIMATES IN SOCIAL RESEARCH

Nonhuman primates are uniquely valuable as models of complex human phenomena because they are closer to humans in evolutionary history, brain structure/function, and social structure and organization. Early studies in monkeys and apes demonstrated dramatically the profound effects of altered early social experience on later individual and social behavior, and on adult behavioral and reproductive competence (Harlow et al., 1965). Later work, using maternal separation in young monkeys, demonstrated not only immediate behavioral responses to separation, but significant endocrinological and immunological consequences as well (Suomi, 1997). Studies emphasizing alterations in behavioral and physiological development can now be expanded to include studies of altered development of basic brain mechanisms and potential remediation. Social rearing parameters described below refer primarily to nonhuman primate data, and within the nonhuman primates, primarily to Old World monkeys, which have been the most extensively studied, and for which most data are available. Atypical early experience in primates usually results in the appearance of species atypical behaviors. Such behaviors may reflect adaptive changes, rather than pathological, in psychological development. Primates raised with absent or deviant social experience will develop very differently from those raised with species-appropriate experience (Bayne and Novak, 1998), but such altered developmental trajectories, while differing behaviorally from species-typical behaviors, need not be equated with stress.

CONSPECIFIC

Social primates have the highest probability of developing in a species-typical manner if reared in a social environment modeled after those found in the wild. This may be especially important when a research program requires subjects typical of those found in the wild, because lab-reared individuals may vary in behavioral characteristics.

PEER REARING

Monkeys raised only with peers may develop sufficient social skills to permit their introduction to more species-typical social groups later in life, but their social repertoires remain somewhat atypical. Typically, peer-rearing paradigms include removing infants from their mothers within 24 to 48 hours of birth, placing them in a temperature- and light-controlled environment, hand feeding them until they are able to nurse from a bottle unsupported, and placing them with a similar-age peer within the first week or two of life. Peer-reared animals will develop strong attachments to each other, and protest vigorously when separated from each other, but the physiological response to separation from a peer is not as profound as is separation from the mother (Boccia et al., 1989).

SURROGATE AND ISOLATION REARING

Surrogate-reared animals are also separated from their mothers shortly after birth, and like peer-reared animals, they are fed by hand until they are able to feed themselves. Instead of

being placed with a peer, they can be provided with a variety of cloth or other surrogates (depending upon experimental issues) in their cage. Physiological development appears to proceed normally in surrogate-reared infants (Reite et al., 1978). They will evidence an apparent strong attachment to their surrogate and will protest vigorously if separated from it, but the physiological consequences of separation from the surrogate are minimal and are not as profound as the consequences of peer or maternal separation (Reite et al., 1989). If provided human contact, they will also form close bonds with their human caretakers, which must be under experimental control. In the absence of appropriate social experience, these animals will develop highly species-atypical social repertoires, effectively precluding their later integration into social groups. This fact must be considered in planning for the animals following their completion of nonterminal experimental paradigms. Rhesus monkeys have been raised with other species, such as mongrel dogs, and in this environment have been shown to develop more species-typical social behavior. Thus social experience need not be with a conspecific, although social behavioral development may be skewed (Mason and Kenney, 1974; Woolverton et al., 1989).

ALTERATIONS IN PARENTING BEHAVIOR

Modifications (usually deficiencies) in parenting behavior can be unwanted by-products of other social or behavioral interventions, or they may be the primary subject of research. Primates raised in social isolation or deprivation may be poor parents (Reite, 1987; Woolverton et al., 1989). Similarly, animals subject to crowding or lack of social support may exhibit abuse of their own infants. ■

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CHAPTER 9

Ethological Approaches

Ethology is the study of species-typical patterns of behavior—with a focus on uncovering the causes, function, development, and evolutionary significance of such behavior. (See Novak et al., 1998, for a more detailed examination of this topic.) Ethological research differs from most behavioral research in that the animal is neither a model nor a surrogate for another species. Ethology includes a wider range of species. For many of these species, there is little information on optimal housing and husbandry. Instead, unique environments are designed by the researcher to elicit and maintain the behavior patterns of interest. Such environments frequently require alterations in husbandry practices. The ILAR Report (ILAR, 1996) permits naturalistic environments. In some instances, however, IACUC approval of exceptions may be required. The sections below identify possible welfare issues pertaining to ethological research.

PASSIVE OBSERVATION

Some ethologists study animals to learn about habitat utilization, foraging strategies, breeding patterns, and social organization. Care should be taken to minimize harmful effects of the observation process on other populations living in the setting or being a vector of disease, thereby increasing the risk of predation in prey species or reducing capture rates in predatory species.

Difficulty in observing a free-ranging population may require provisioning (augmenting the natural food supply) to bring animals close to the observer. The provisioned material should minimize possible dietary imbalances. The subject population may be exposed to models or to other living animals, or their odors or vocalizations. Because provisioning may artificially increase population densities, the researcher must be alert to heightened aggression and ultimately lowered reproduction. When the study is over, loss of provisioning may result in a higher mortality because the environment can no longer support the expanded population. These effects may be partially controlled by considering the frequency and length of the provisioning period as well as the actual distribution of food in terms of the area covered.

Whenever the habitat is altered, there may be changes in breeding rates or in the risk of predation. When the exposure involves a living animal, special techniques may be required

for protecting the stimulus and the subject population from one another (e.g., holding cages). Additional attention should be paid to the stimulus animal's social status if it is a conspecific. Once the exposure is over, the stimulus animal must either be returned to its original location or be incorporated into the subject population. Novak et al. (1998) describe methods for capture, sedation, and marking of free-ranging animals.

ENCLOSURES

A number of species are housed in large groups in enclosures outdoors (e.g., ungulates, rodents, and canids), in zoological parks, or in laboratories (e.g., nonhuman primates). Observation of these animals may occur from blinds, catwalks, or other areas that are separated from the animals, or the observers may move freely among the animals. When observers and animals can intermingle, there are risks to the health and welfare of both animals and observers. Thus, observers should be knowledgeable about the behavior of the species they are observing. For example, they should be aware of flight distances and not inadvertently corner animals. Before they are allowed to observe animals independently, they should receive training from experienced, on-site personnel on how to respond to particular individuals and particular situations and how to protect themselves from danger. Observers need to be screened for the presence of diseases that may be highly transmissible to the animals. They should also receive prophylactic inoculations and tests (e.g., against rabies, tuberculosis) where relevant.

Animals housed in large social groups require planning for their separation from the group if they become ill or injured, and for the return to the group. In some primate species, such re-introductions can be problematic depending on the animal's sex and rank, the length of the time away from the group, and the initial cause of the removal.

Ethologists often incorporate key ecological elements into their laboratories. Arboreal species are usually given access to climbing surfaces and structures; scent-marking species are provided with relevant marking surfaces that are not sanitized in every cleaning cycle; and burrowing species are housed under natural covers such as hay.

Sanitation objectives need not conflict with "naturalizing" the animal's environment (e.g., items made of wood should be spot cleaned and removed when worn). For some rodent species, the transfer of a small amount of soiled bedding to clean cages may actually improve reproductive success. Furthermore, scent-marking surfaces should not be routinely cleaned because this often creates the situation of a "strange environment," and for some animals the result is excessive scent-marking behavior and physiological stress.

WILD-CAUGHT ANIMALS AS RESEARCH SUBJECTS

Wild-caught animals are studied in captivity to observe behavior under controlled conditions.

Appropriate permits must be obtained for the live capture and subsequent use of animals in captivity. Typically, wild-caught animals have internal and external parasites. Quarantine of newly arrived animals is needed to protect the health of those already in the colony, to determine the health status of the incoming animals, and to safeguard the health of personnel. The quarantine also allows the animal's metabolism to adjust to the new environmental conditions and gives the animal time to recover physiologically, immunologically, and behaviorally from the stress of capture and transplantation.

An important concern for those working with wild-caught animals is the final disposition of the animal after experiments are completed. At least three options may be relevant, including euthanasia, placement in another research facility, or the return of the animals to their natural habitat. Resolution of this issue depends on a number of practical as well as ethical concerns. If the animal is to be returned to its native environment, the following should be considered: (1) the likelihood of the animal's readjusting to nature, with time in captivity as one relevant marker; (2) the specific environment to which it may be returned (i.e., the same or similar?); and (3) the possible impact on that environment. Because all three options have costs and benefits depending on the species and the circumstances, it may be necessary to determine the fate of wild-caught animals on a study-by-study basis. These issues should be addressed during the permit application process. Information on social manipulation can be found in Chapter 8, Social Variables (see also Novak et al., 1998).

Research on infanticide examines the response of adults to young offspring to make inferences about social organization and patterns of parental care. This research often entails injury or death to neonates and thus is problematic because of the high probability of pain and distress. Offspring can be placed in a protective barrier (e.g., mesh cage) to reduce the potential for injury from adults. Aggression toward offspring in mesh cages is then used in place of actual killing of offspring. In some species, however, this procedure inhibits the infanticide response. Extensive observation can reduce the probability of injury. Adults are observed closely for behavioral signs of imminent attack (e.g., lunges in rodents). When these signs are observed, the adult is then distracted or removed from the testing environment before killing occurs.

Studies of predator/prey relationships can provide clues to the animal's ecological niche, cognitive capacity, sensory capacity, and adaptations as a predator or as prey. Such work also provides insights into the neural mechanisms of aggression when coupled with standard neurophysiological and neuropharmacological procedures. A major welfare issue is the occurrence of pain and injury. The prey species is usually the one at risk for injury. It is sometimes possible to protect prey from physical attack with the use of holding cages. However, this procedure is useful only if predators continue to make predatory moves under such conditions. Modeling aspects of the predation sequence can sometimes eliminate risk of

injury in the prey. For example, prey recognition must occur before the predatory sequence is fully initiated. In many cases, it is not necessary to use live prey for studying this facet of predation. This strategy cannot be used when movement of the prey is necessary both for recognition and for predatory behavior. Although injury is a primary concern for prey, it should also be noted that prey animals may harm predators.

One should consider limits on the number of times an animal serves as a prey based on changes in stimulus behavior or signs of accumulating stress. Furthermore, prey that are wild-caught generally have more experience with predators than laboratory animals and may provide a more accurate portrayal of the true sequence of events. Using a laboratory mouse rather than a field mouse as prey for a carnivore, for example, may not generate a true-to-life rendition of the escape strategies employed by the prey and the counterstrategies used by the predator. Similar arguments can be advanced for the predator. ■

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Internet links to field study guides:

American Society of Mammologists
http://asm.wku.edu/committees/animal_care_and_use/98IACUCguidelines.PDF

American Ornithologists Union
http://www.nmnh.si.edu/BIRDNET/GuideToUse/index.html

Guidelines for Use of Fishes in Field Research
http://www.utexas.edu/depts/asih/pubs/fishguide.html

Guidelines for Use of Live Amphibians and Reptiles in Field Research
http://www.utexas.edu/depts/asih/pubs/herpcoll.html

Guidelines for Use of Live Amphibians and Reptiles in Field Research
http://www.utexas.edu/depts/asih/pubs/herpcoll.html

CHAPTER 10

Teaching with Animals

Understanding of biological and experiential influences on behavior is furthered by studies of live subjects. In order to improve on what we know now, new students must be inspired to carry these investigations into the next generation of Behavioral Science. We cannot rely on simulations to encourage such reevaluation or to challenge students. Computer simulations, like written descriptions, provide only a brief, almost cartoon-like sketch of what we know. Students tend to treat their time with simulations as "practice" rather than as an encounter with the subject matter. Simulations may be the best approach for training in a particular procedure or merely a review of what is known about a subject. On the other hand, work with live subjects is superior if the project seeks to pique student interest, to encourage students to critically evaluate established or emerging ideas, or to help students rise to the challenge of creating new ideas about biological and experiential influences on behavior.

One must be straightforward about the many issues that need to be addressed as educational projects are developed, approved for use, and carried out. Statements issued by professional and governmental agencies are useful to frame what is and what is not judged appropriate for such educational projects. Painful or stressful studies should not be performed for educational purposes alone.

The United States Congress Office of Technology Assessment (OTA, 1986) has identified the following goals for the educational use of animals:

> (1) Development of positive attitudes toward animals. In the best instances, such development incorporates ethical and moral considerations into the student's course of study. (2) Introduction of the concept of biological models, by which students learn to single out particular animal species as representative of biological phenomena. Such models vary in the degree to which they provide general information about a broader spectrum of life. (3) Exercise of skills vital to intellectual, motor, or career development. Familiarity with living tissue, for example, enhances a student's surgical dexterity.

The guidebook for IACUCs, recently revised by the Applied Research Ethics National Association (ARENA) and the Office for Laboratory Animal Welfare (OLAW) (2001), makes the following statement on educational uses of animals: "All instructional proposals should clearly identify the learning objectives and justify the particular value of animal use as part of

the course, whether it is demonstration of a known phenomenon, acquisition of practical skills, or exposure to research."

Common sense and sensitivity on the part of the teacher and the IACUC should ensure that animals are used appropriately and that interested students are not deprived of educational opportunities. Instructors and the IACUC should work together in developing institutional guidelines that maximize learning opportunities and the welfare of the animals used. Cunningham, Panicker, and Akins (in preparation) inform college and university instructors about Federal guidelines and policies for the use of animals in teaching as well as instructional projects that have been used successfully.

Tait (1993) has suggested several questions that the instructor may find helpful to consider when preparing an exercise involving undergraduate students: (1) What is the pedagogical purpose of the proposed protocol? (2) At what academic level are the students? (3) What are the future prospects of the students—do the students have a high degree of commitment to the discipline? (4) Are alternatives such as video or computer simulation available, and would they be equally effective? (5) Who will prepare the animals for the experience? ■

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CHAPTER 11

Resources for Further Information

AMERICAN ASSOCIATION FOR LABORATORY ANIMAL SCIENCE (AALAS)
70 Timber Creek Drive
Cordova, TN 38018-4233
Phone: (901) 754-8620
Fax: (901) 753-0046
E-mail: info@aalas.org
Web site: http://www.aalas.org/

AMERICAN COLLEGE OF LABORATORY ANIMAL MEDICINE (ACLAM)
Dr. Melvin W. Balk
96 Chester Street
Chester, NH 03036
Phone: (603) 887-2467
Fax: (603) 887-0096
E-mail: mwbaclam@gsinet.net
Web site: http://www.aclam.org

ANIMAL WELFARE INFORMATION CENTER (AWIC)
10301 Baltimore Avenue
Beltsville, MD 20705-2351
Phone: (301) 504-5755
E-mail: webmaster@nal.usda.gov
Web site: http://www.nal.usda.gov/awic/awic.htm

APPLIED RESEARCH ETHICS NATIONAL ASSOCIATION (ARENA)
132 Boylston Street, 4th floor
Boston, MA 02116
Phone: (617) 423-4112
Fax: (617) 423-1185
E-mail: prmr@aol.com
Web site: http://www.aamc.org/research/primr/arena

ASSOCIATION FOR ASSESSMENT AND ACCREDITATION OF LABORATORY ANIMAL CARE, INTERNATIONAL (AAALAC)

11300 Rockville Pike, Suite 1211
Rockville, MD 20852-3035
Phone: (301) 231-5353
Fax: (301) 231-8282
E-mail: accredit@aaalac.org
Web site: http://www.aaalac.org

INSTITUTE FOR LABORATORY ANIMAL RESOURCES (ILAR)

2101 Constitution Avenue, NW
Washington, DC 20418
Phone: (202) 334-2590
Fax: (202) 334-1687
E-mail: ILAR@nas.edu
Web site: http://www2.nas.edu/ilarhome/

OFFICE OF LABORATORY ANIMAL WELFARE (OLAW)

National Institutes of Health

RKL1, Suite 1050
MSC7982
Bethesda, MD 20892-7982
Phone: (301) 594-2382
Fax: (301) 402-2803
Web site: http://www.nih.gov/grants/olaw/olaw_t.htm

SCIENTISTS CENTER FOR ANIMAL WELFARE (SCAW)

7833 Walker Drive, Suite 340
Greenbelt, MD 20770
Phone: (301) 345-3500
E-mail: scaw@erols.com
Web site: http://www.scaw.com

UNITED STATES DEPARTMENT OF AGRICULTURE (USDA)

Animal and Plant Health Inspection Service
Riverdale, MD 20737
Phone: (301) 336-5953
E-mail: ace@usda.gov
Web site: http://www.aphis.usda.gov/reac/

Some relevant scientific societies with animal care committees:

AMERICAN PHYSIOLOGICAL SOCIETY
9650 Rockville Pike
Bethesda, MD 20814-3991
Phone: (301) 530-7164
E-mail: webmaster@aps.faseb.org
Web site: http://www.faseb.org/aps/

AMERICAN PSYCHOLOGICAL ASSOCIATION
Science Directorate
750 First Street, NE
Washington, DC 20002
Phone: (202) 336-5500
E-mail: science@apa.org
Fax: (202) 336-5953
Web site: http://www.apa.org/

AMERICAN VETERINARY MEDICAL ASSOCIATION
1931 North Meacham Road, Suite 100
Schaumburg, IL 60173
Phone: (847) 925-8070
Fax: (847) 925-1329
E-mail: AVMAINFO@avma.org
Web site: http://www.avma.org/

FEDERATION OF ANIMAL SCIENCE SOCIETIES
1111 North Dunlap Avenue
Savoy, IL 61874
Phone: (217) 356-3182
FAX: (217) 398-4119
E-mail: fass@assochq.org
Web site: http://www.fass.org

SLEEP RESEARCH SOCIETY
6301 Bandel Road, Suite 101
Rochester, MN 55901
Phone: (507) 287-0846
Web site: http://www.srssleep.org/

SOCIETY FOR NEUROSCIENCE
11 Dupont Circle, NW, Suite 500
Washington, DC 20036
Phone: (202) 462-6688
E-mail: info@sfn.org
Web site: http://www.sfn.org

SOCIETY OF TOXICOLOGY
1767 Business Center Drive, Suite 302
Reston, VA 22090
Phone: (703) 438-3115
Fax: (703) 438-3113
E-mail: sothq@toxicology.org
Web site: http://www.toxicology.org

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